Characterization of BBa_F2620,

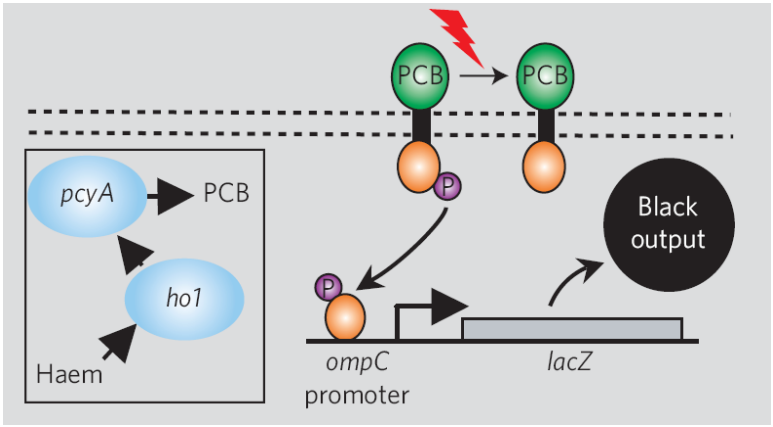
an engineered cell-cell communication device

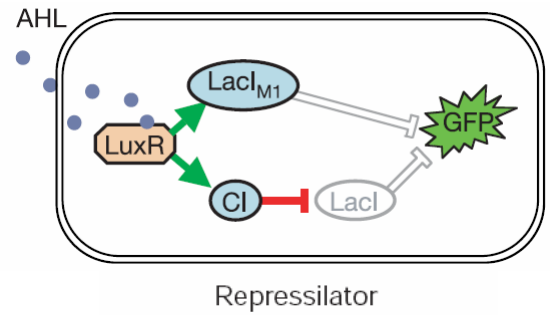
Anna Labno

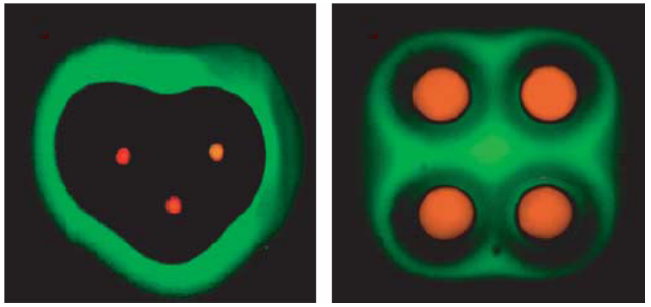
Barry Canton

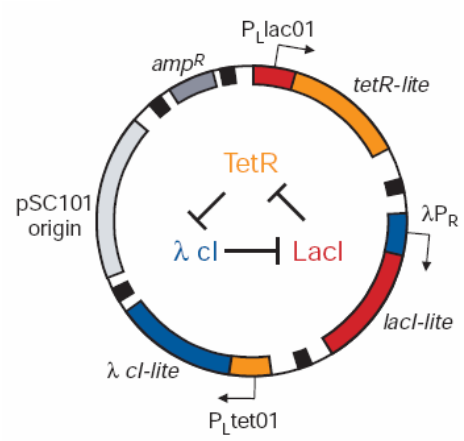
Drew Endy

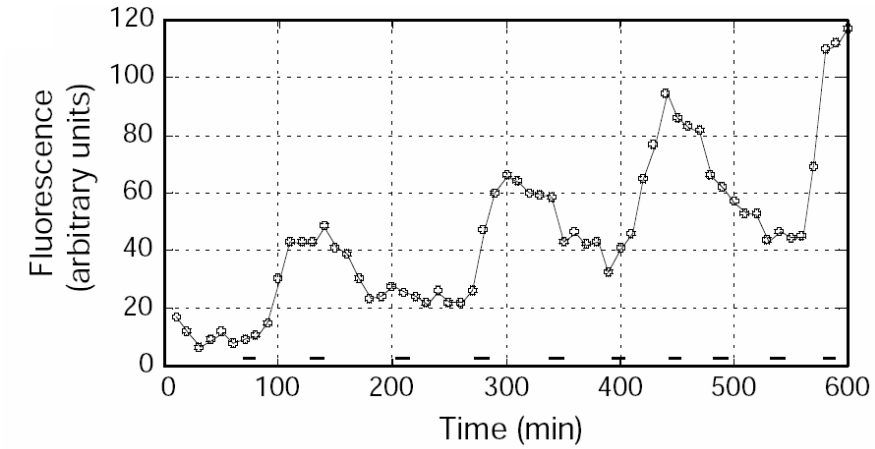
Massachusetts Institute of Technology







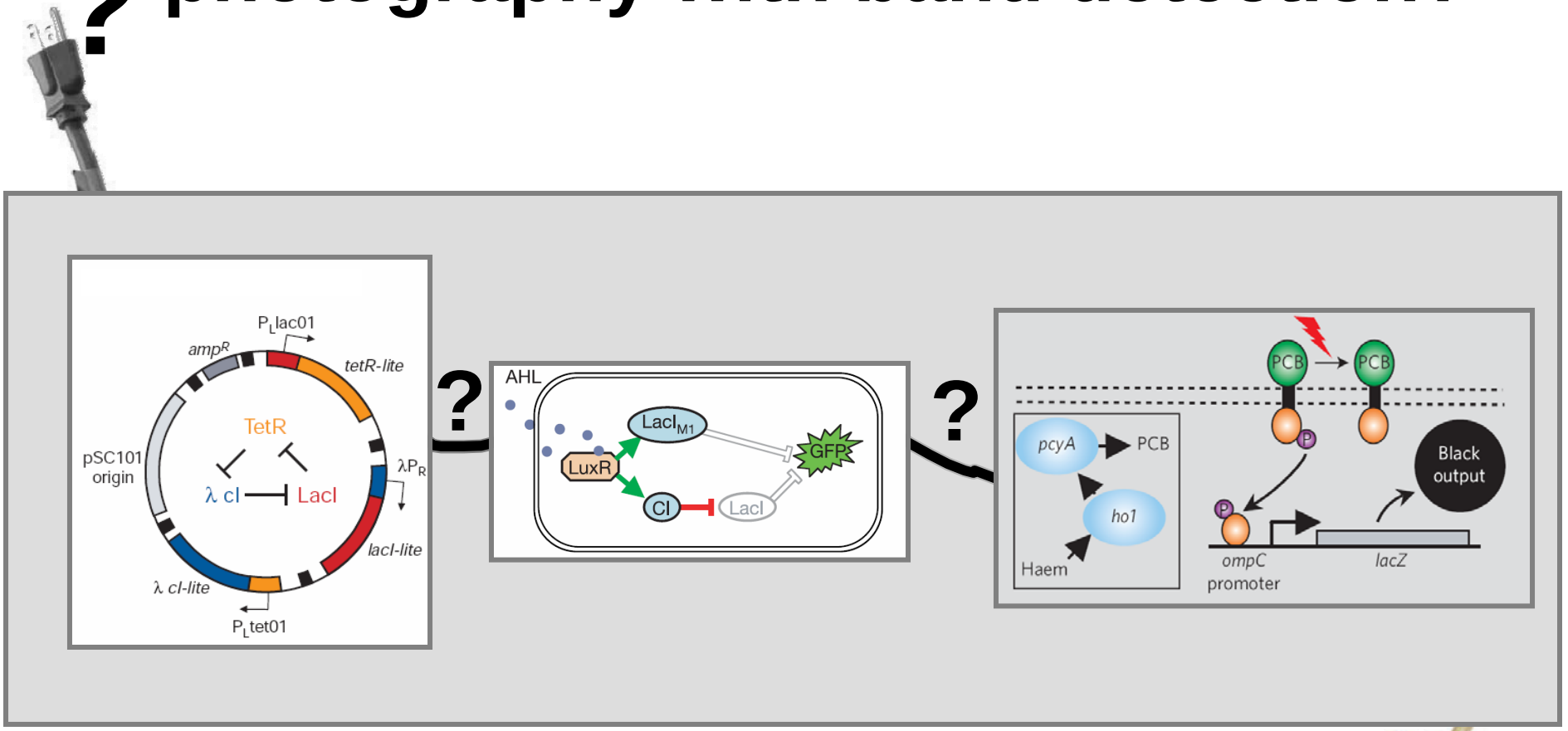




Engineering *Escherichia coli* to see light; Anselm Levskaya at al; A synthetic oscillatory network; of transcriptional regulators Michael B. Elowitz & Stanislas Leibler

A synthetic multicellular system for programmed pattern formation; Subhayu Basu at al

Repressilator controlled photography with band detection?



In most engineering disciplines…

Can we make biology like this?

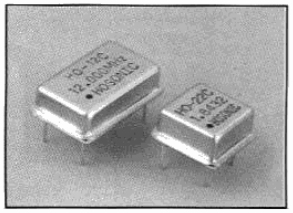
HCMOS/TTL Crystal Oscillators — HOSONIC

Full Size 12 Series And Half Size 22 Series

PART NUMBERING SYSTEM

D HO-12 C-10.000—S—XX

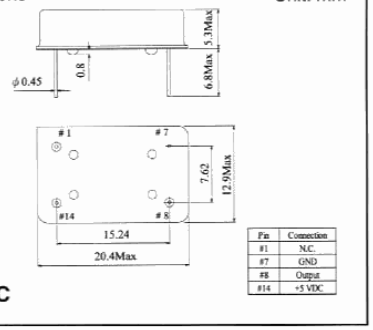
- Hosonic X'tal Oscillator
- Model NO. (12:Full, 22:Half)
- Frequency Stability (Code)
- Frequency (MHz)
- Available Options
- Temperature Range
  - Blank=0°C to 70°C
  - S=-40°C to 85°C



■ ELECTRICAL CHARACTERISTICS

Model		HO-12 series	HO-22 series
Package		14 Pin DIP (Full Size)	8 Pin DIP (Half Size)
Frequency Range	(Fo)	1.000MHz to 100.000MHz	
Frequency Stability	(△f/fo)	A=±25ppm, B=±50ppm, C=±100ppm	
Operating Temperature Range	(Topr)	0°C to 70°C, -40°C to 85°C (Available)	
Storage Temperature	(Tstg)	-55°C to 125°C	
Power Supply Voltage	(VDD)	5V±0.5VDC	
Aging (at 25°C)		±5ppm/year Max.	
		1.000MHz to 23.000MHz 20mA Max.	
			x.
			x.
			x.
			x.
Rise and Fall Time 10% to 90% of Waveform W/Hcmos Load	(TR, TF)	5ns Max.	
Output Voltage Logic High	(VOH)	4.5V Min.	
Output Voltage Logic Low	(VOL)	0.5V Max.	
Output load	1.000MHz to 40.000MHz	10TTL Load or 50pF HCMOS Load	
	40.000MHz to 100.000MHz	10TTL Load or 15pF HCMOS Load	

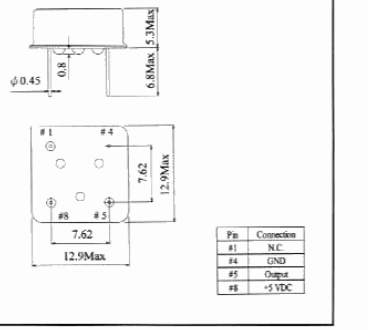
Dimensions — Unit: mm



Pin	Connection
#1	N.C.
#7	GND
#8	Output
#14	+5 VDC

HO-12C

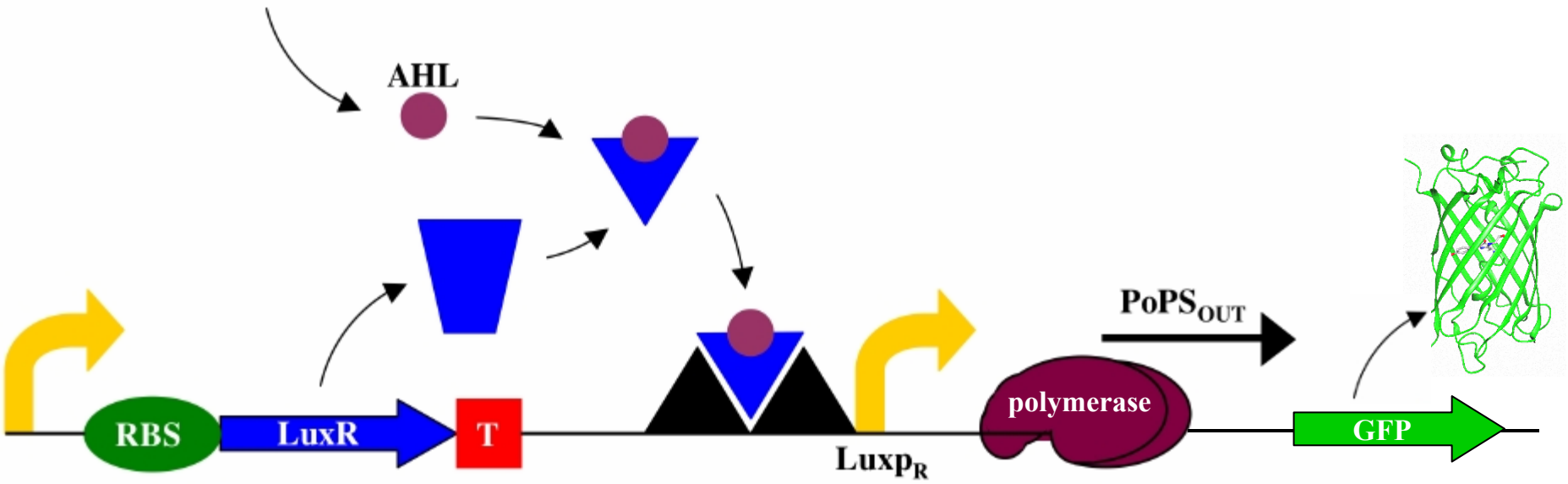
Dimensions — Unit: mm



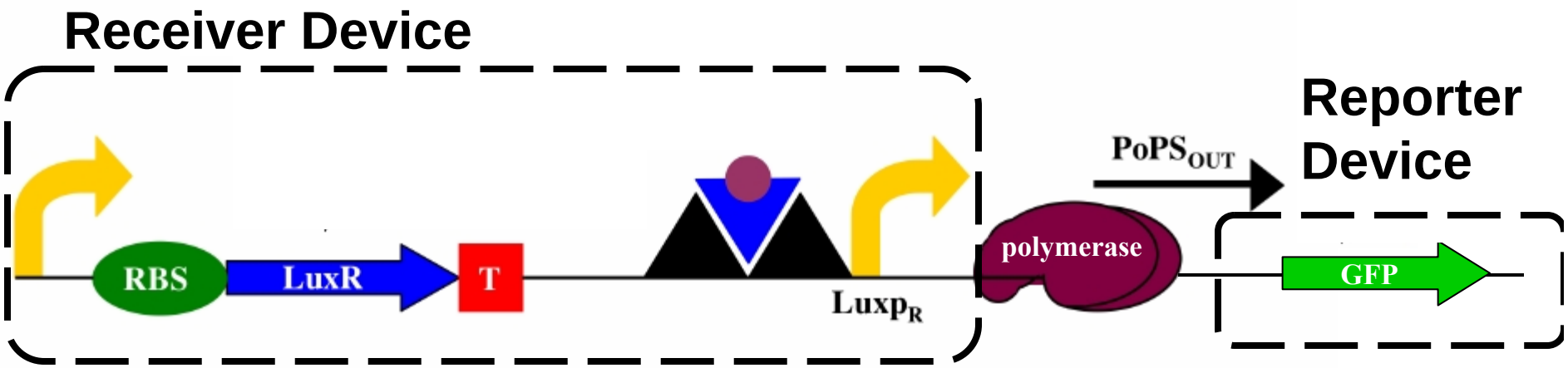
Pin	Connection
#1	N.C.
#4	GND
#5	Output
#8	+5 VDC

HO-22C

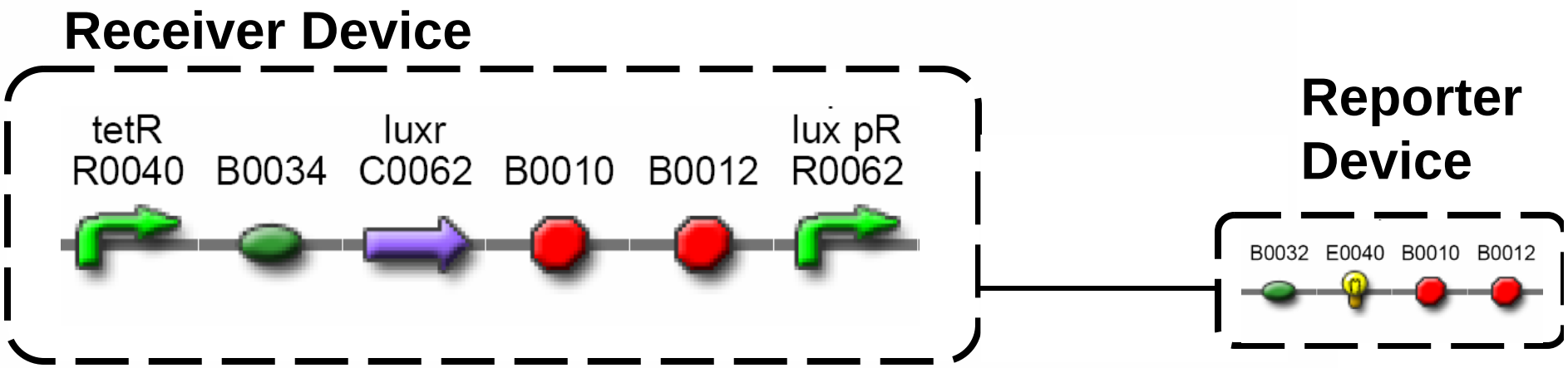
Cell-Cell Communication Device



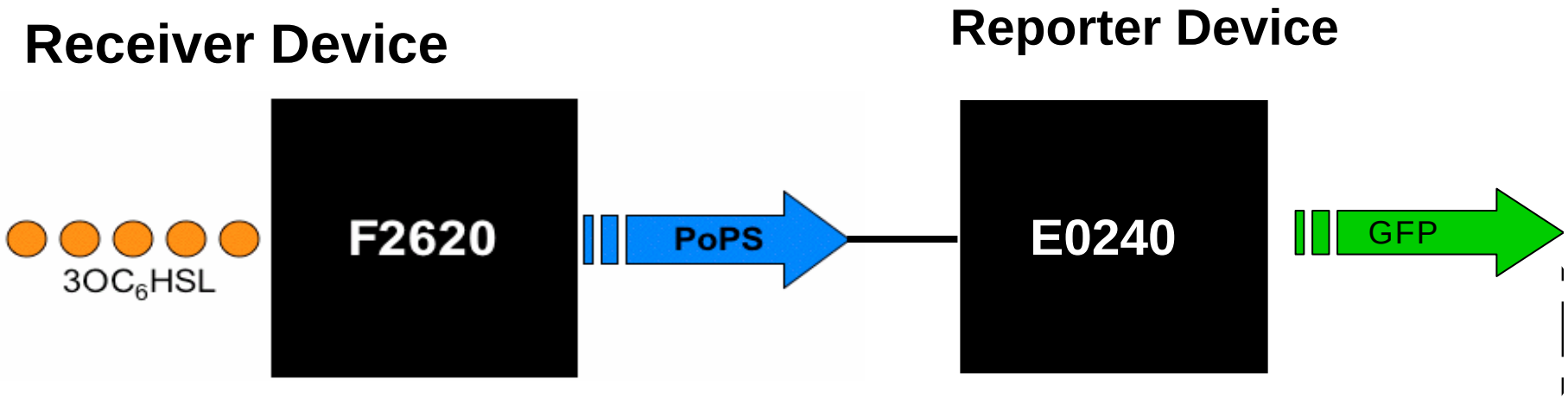
Cell-Cell Communication Device

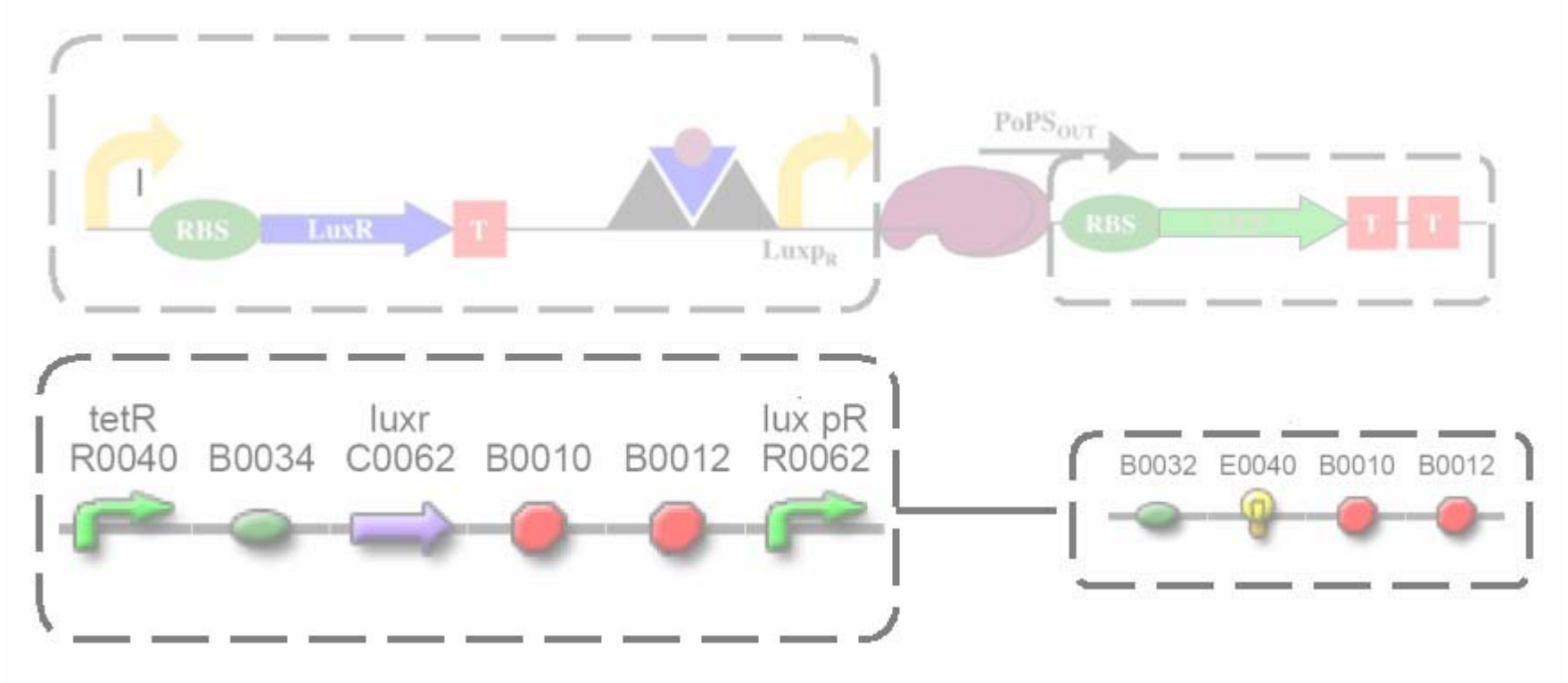


Cell-Cell Communication Device



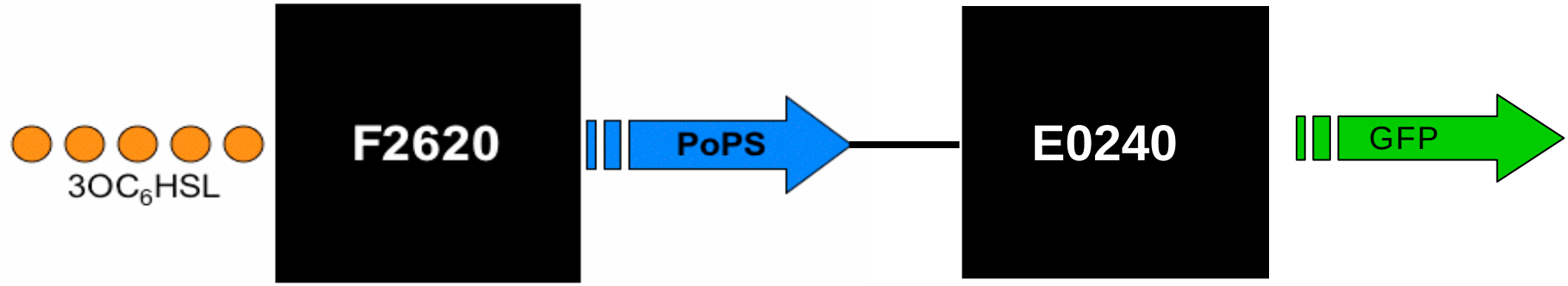
Cell-Cell Communication Device





Receiver Device

Reporter Device

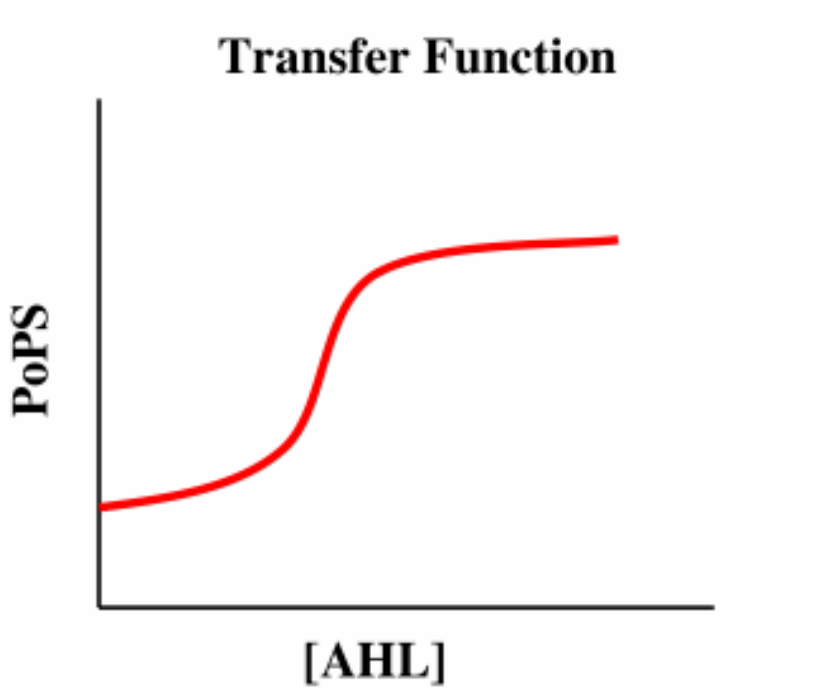


Relevant Device Characteristics

- Transfer Function
- Variability
- Specificity (with respect to inducer)
- Latency (High/Low)
- Stability (genetic and performance)

Relevant Device Characteristics

- Transfer Function



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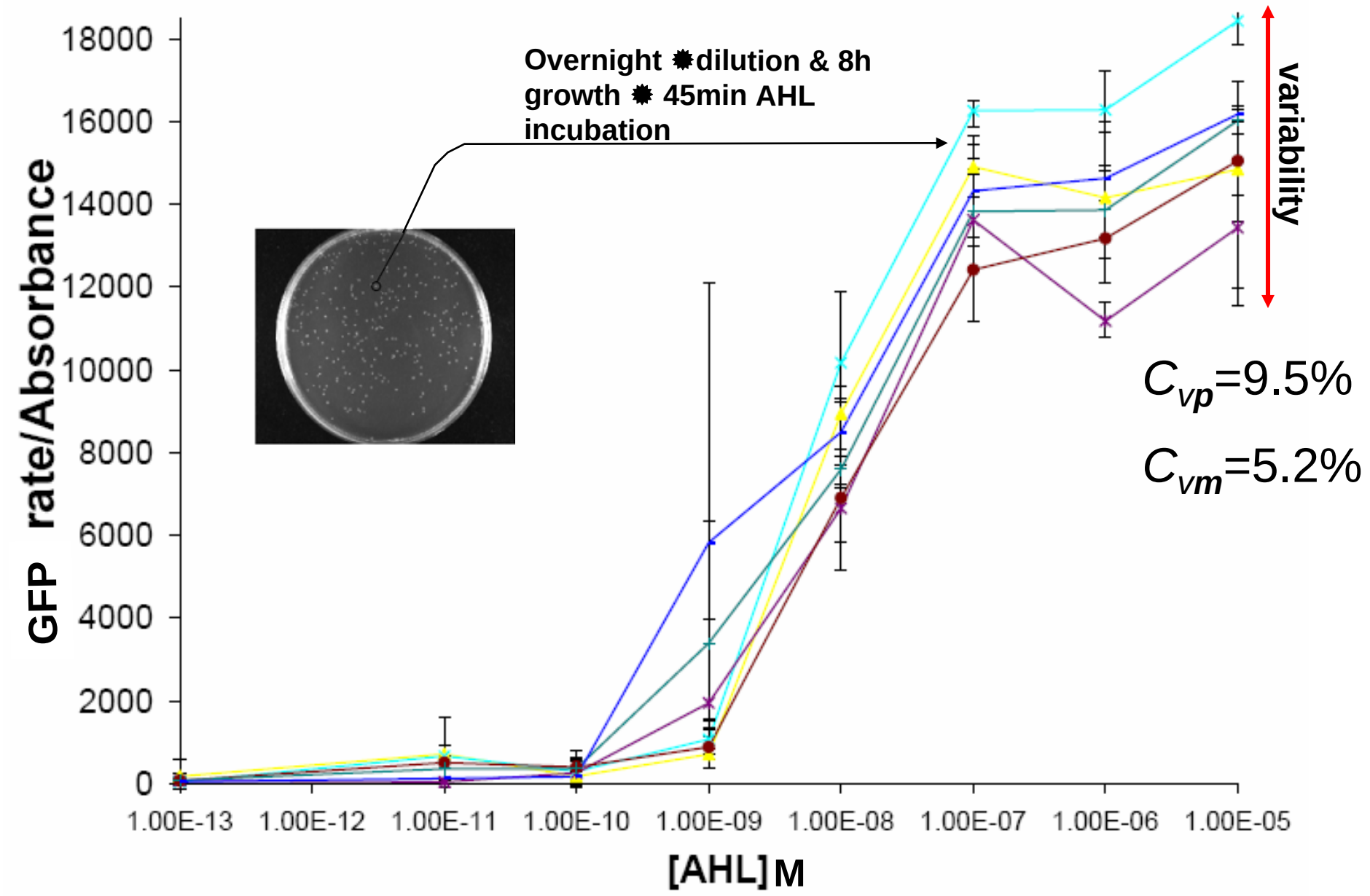
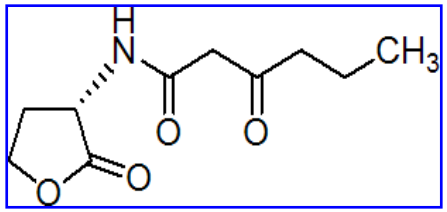
Variability

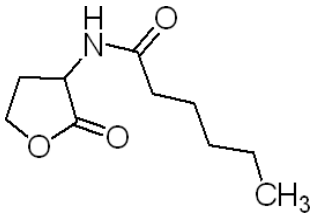


Specificity-Reagents

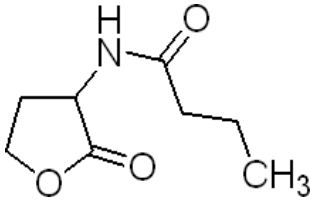
N-(β-Ketocaproyl)-DL-homoserine lactone **(cognate)**



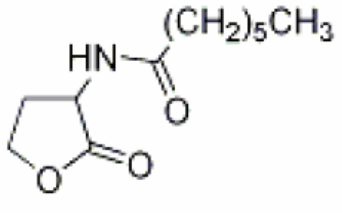
N-Hexanoyl-DL-homoserine lactone



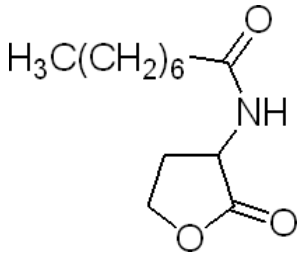
N-Butyryl-DL-homoserine lactone



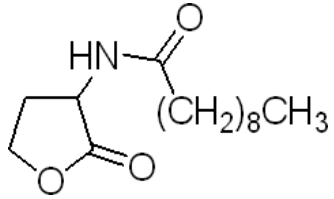
N-Heptanoyl-DL-homoserine lactone



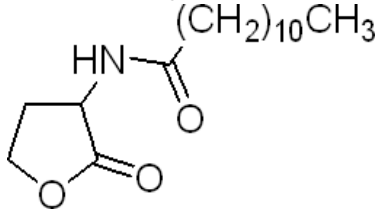
N-Octanoyl-DL-homoserine lactone



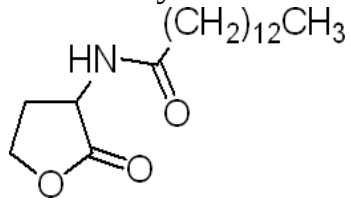
N-Decanoyl-DL-homoserine lactone



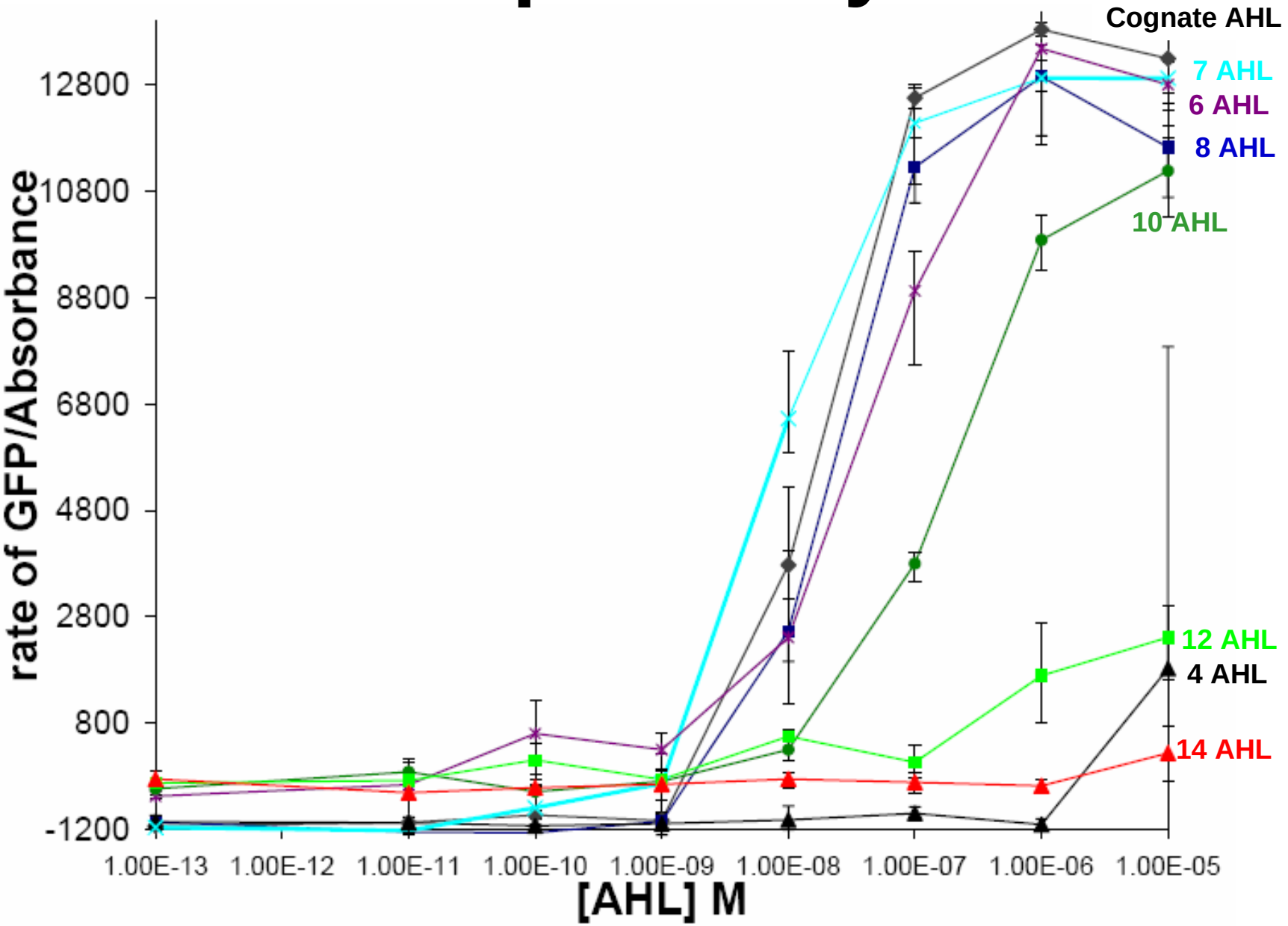
N-Dodecanoyl-DL-homoserine lactone



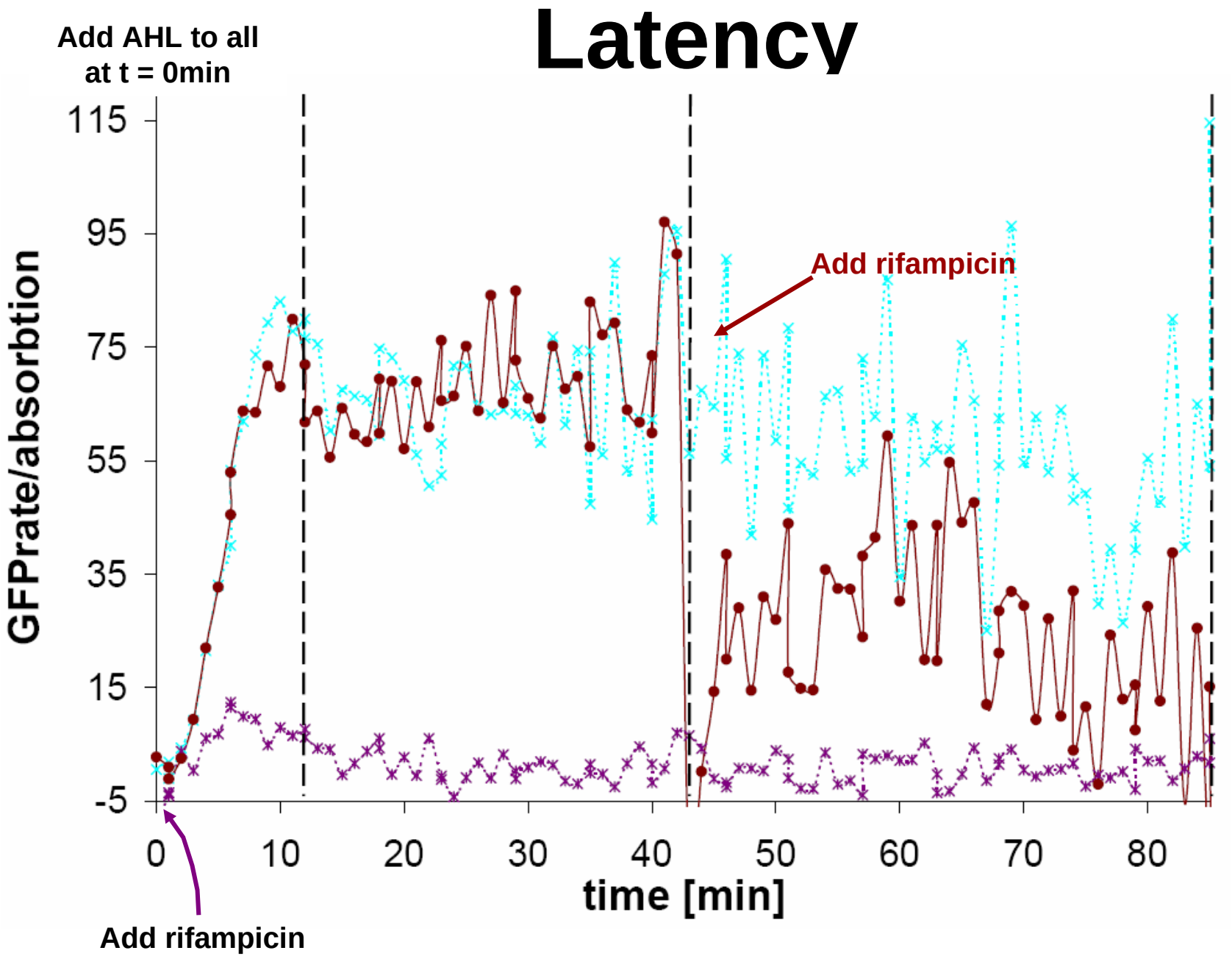
N-Tetradecanoyl-DL-homoserine lactone



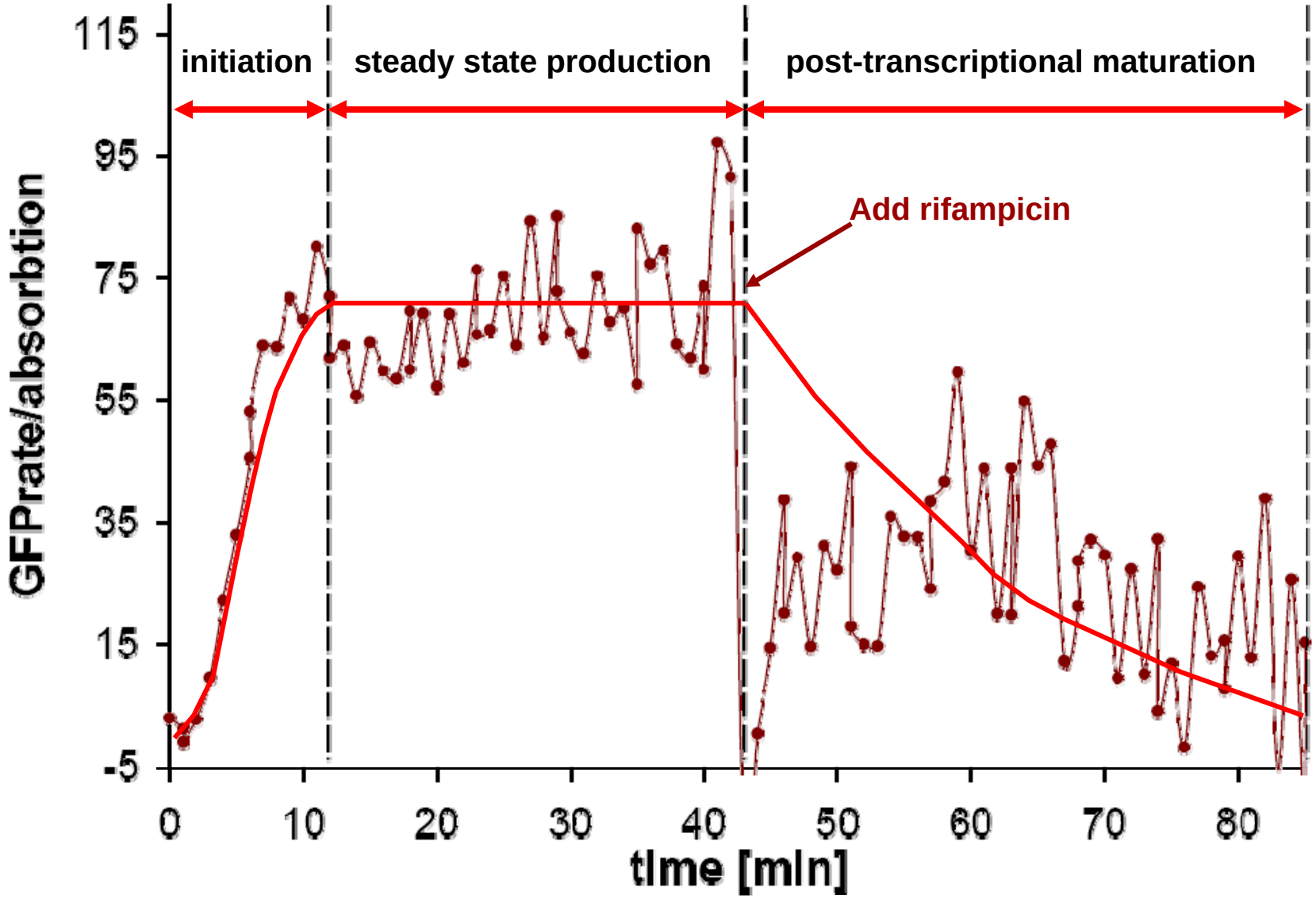
Specificity



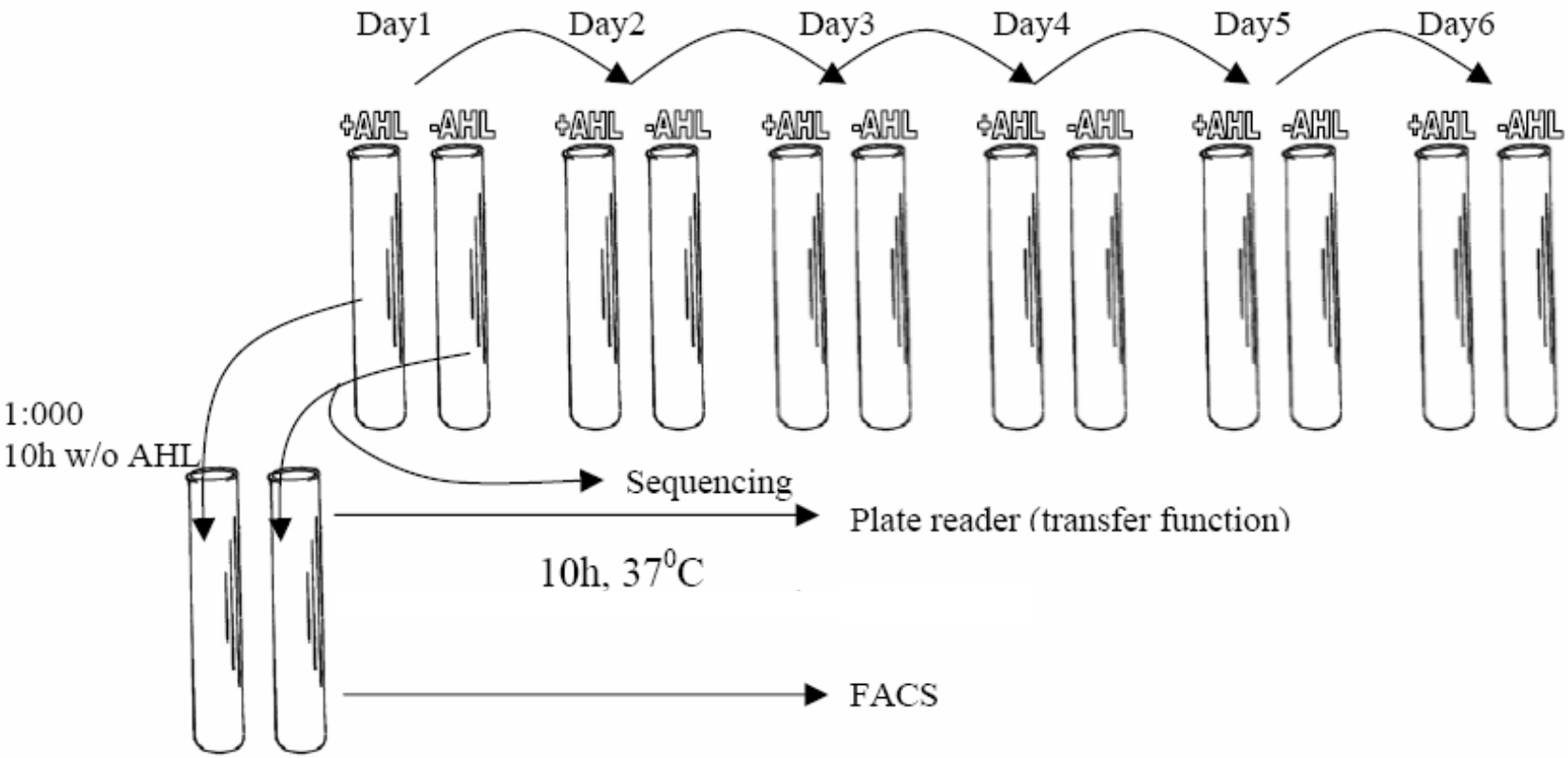
Latency

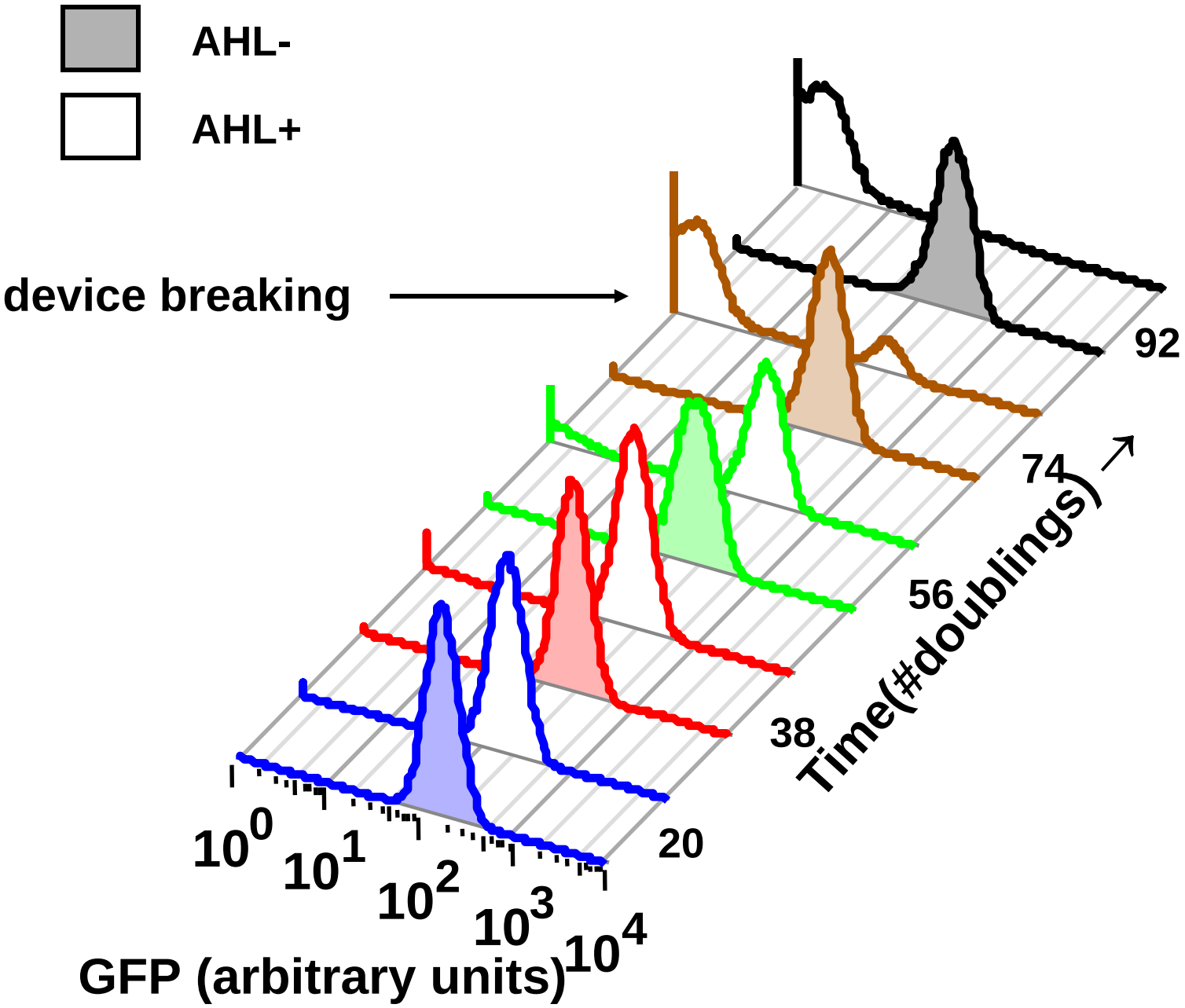

Add AHL to all
at t = 0min
GFPrate/absorbtion
115
95
75
55
35
15
-5
0
10
20
30
40
50
60
70
80
time [min]
Add rifampicin
Add rifampicin

Latency


initiation
steady state production
post-transcriptional maturation
Add rifampicin
GFPrate/absorbtion
115
95
75
55
35
15
-5
0
10
20
30
40
50
60
70
80
time [min]

Device Stability




AHL-
AHL+
device breaking
92
74
56
38
20
Time(#doublings)
10^0
10^1
10^2
10^3
10^4
GFP (arbitrary units)

And now in biology….

BBa_F2620

$3OC_6HSL \rightarrow$ PoPS Receiver

Author(s): Barry Canton [bcanton@mit.edu]

Last Update: March 10, 2006

Description and Usage:

***Device input* is $3OC_6HSL$. *Device output* is PoPS** produced at a LuxR-regulated operator

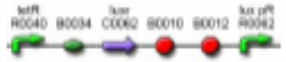
A transcription factor [LuxR] that is active in the presence of cell-cell signaling molecule [$3OC_6HSL$] is constitutively expressed from an operator [TetR].Full PoPS output at high $3OC_6HSL$ levels and low plasmid copy [e.g., pSB3K3] results in a reduced cell growth rate. If used in a cell containing TetR then a second input signal [aTc] can be used to produce a logical *AND* function.

Characteristics

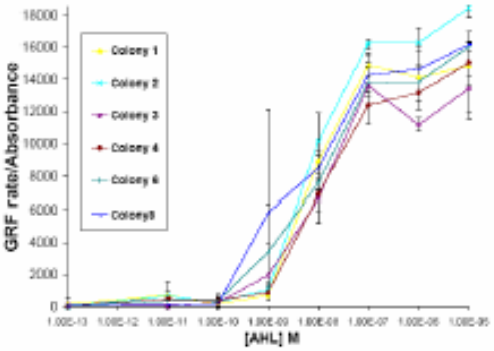
Full Output Variability Coefficient: **9.5%**
Switch Point: **10 nM** $3OC_6HSL$, exogenous
LH Latency: **12** minutes
HL Latency: **40** minutes
Cross Talk: with **AHL derivatives**. See specificity below

Key Components

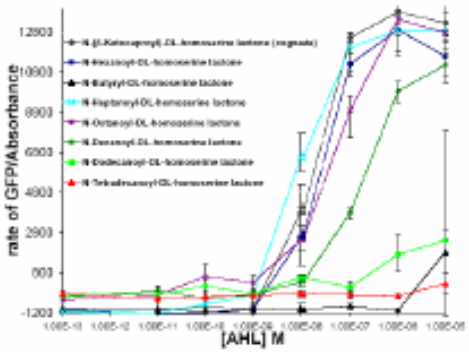
BBa_R0040: TetR-regulated operator
BBa_C0062: luxR ORF
BBa_R0062: LuxR-regulated operator



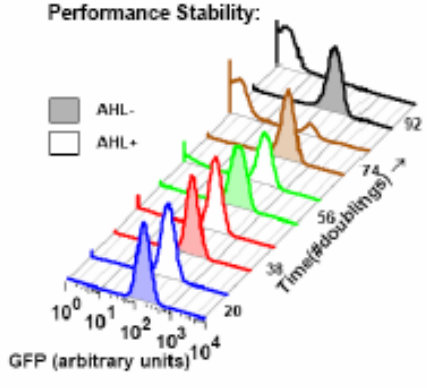
Transfer Function Variability:



Specificity:



Performance Stability:



Full Induction: device non-functional after 74 doublings

No induction: device functional for over 100 doublings

Compatibility

Device has been shown to work in *MC4100*, *MG1655*, and *DH-5α*.

Device has been shown to with on *pSB3K3* and *pSB1A2*.

Device has been shown to work with *E0430* and *E0434*.

Crosstalk with systems containing *TetR*.

*Device output measured indirectly via PoPS-driven fluorescence from *BBa_E0430*. [ ] = geometric mean, arbitrary units. Host cell MC4100, device carried on *pSB3K3*, 100ml batch flask, supplemented M9 media, FACScan cytometer [see *MIT SBWG FACS protocol*].

Registry of Standard Biological Parts
making life better, one part at a time

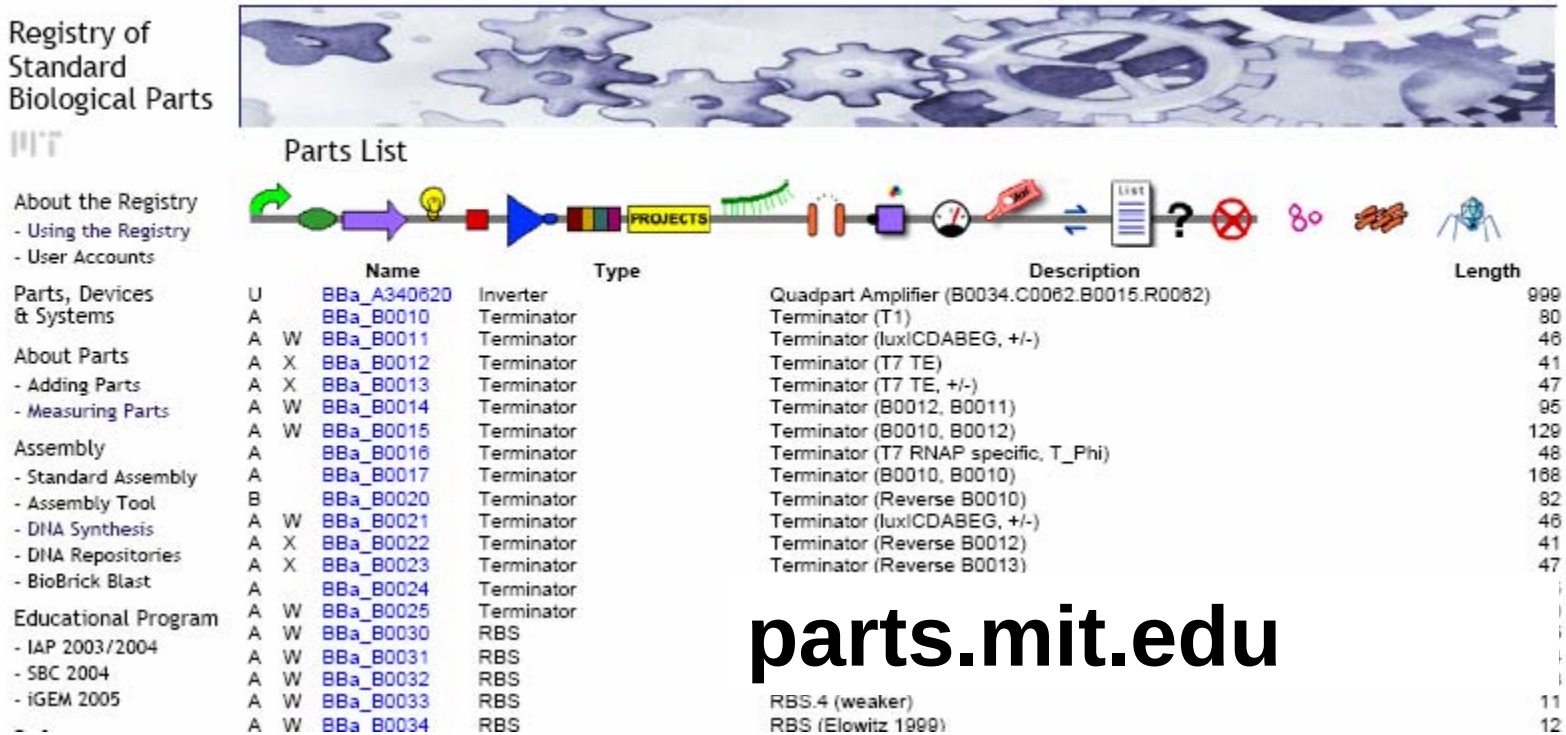
Signaling Devices

Thank You

Massachusetts Institute of Technology

Backup Slides

Registry of Standard Biological Parts



Registry of Standard Biological Parts

MIT

Parts List

PROJECTS

About the Registry
- Using the Registry
- User Accounts

Parts, Devices & Systems

About Parts
- Adding Parts
- Measuring Parts

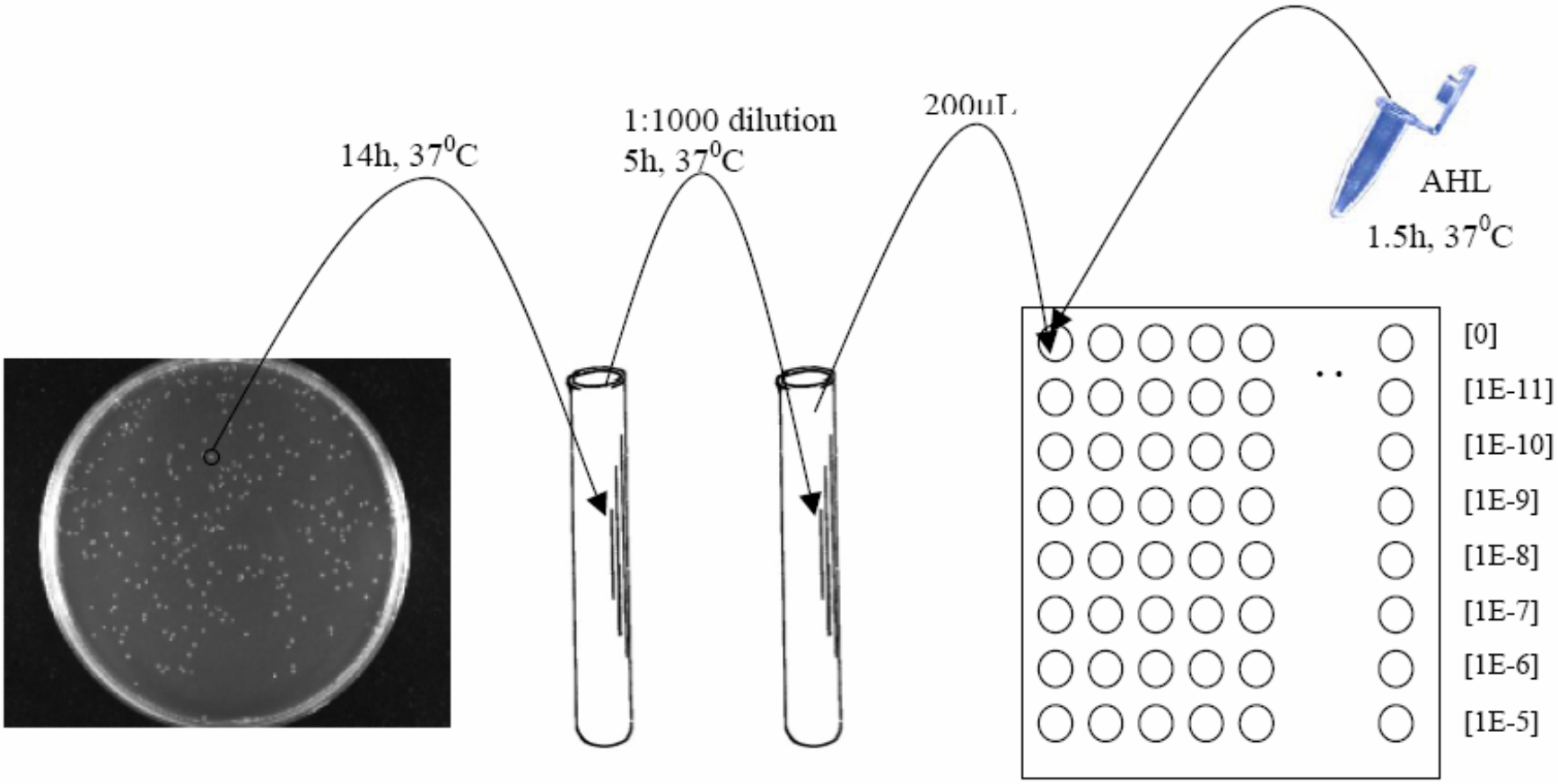
Assembly
- Standard Assembly
- Assembly Tool
- DNA Synthesis
- DNA Repositories
- BioBrick Blast

Educational Program
- IAP 2003/2004
- SBC 2004
- iGEM 2005

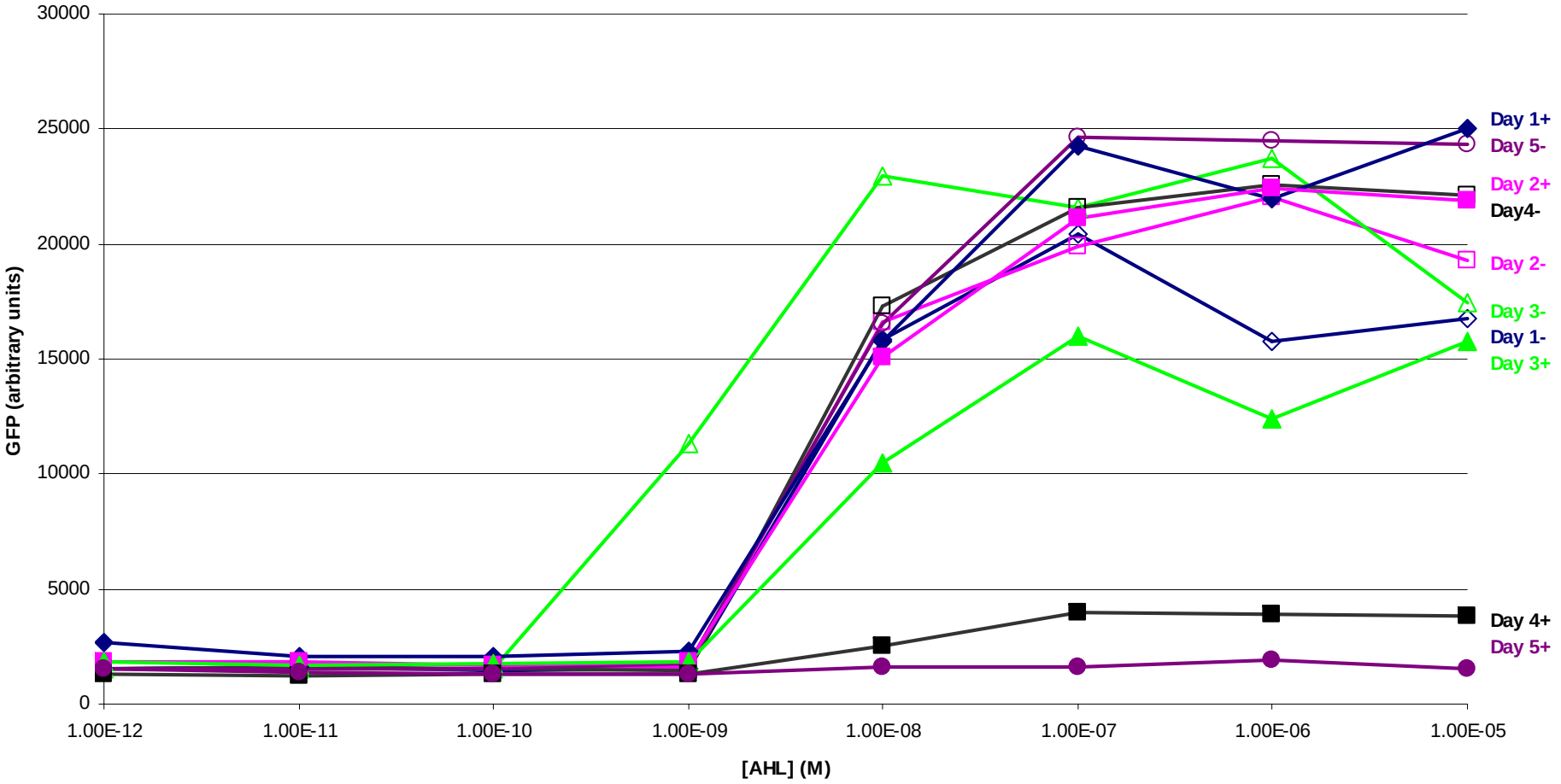
		Name	Type	Description	Length
U		BBa_A340620	Inverter	Quadpart Amplifier (B0034.C0062.B0015.R0062)	999
A		BBa_B0010	Terminator	Terminator (T1)	80
A	W	BBa_B0011	Terminator	Terminator (luxICDABEG, +/-)	46
A	X	BBa_B0012	Terminator	Terminator (T7 TE)	41
A	X	BBa_B0013	Terminator	Terminator (T7 TE, +/-)	47
A	W	BBa_B0014	Terminator	Terminator (B0012, B0011)	95
A	W	BBa_B0015	Terminator	Terminator (B0010, B0012)	129
A		BBa_B0016	Terminator	Terminator (T7 RNAP specific, T_Phi)	48
A		BBa_B0017	Terminator	Terminator (B0010, B0010)	168
B		BBa_B0020	Terminator	Terminator (Reverse B0010)	82
A	W	BBa_B0021	Terminator	Terminator (luxICDABEG, +/-)	46
A	X	BBa_B0022	Terminator	Terminator (Reverse B0012)	41
A	X	BBa_B0023	Terminator	Terminator (Reverse B0013)	47
A		BBa_B0024	Terminator		
A	W	BBa_B0025	Terminator		
A	W	BBa_B0030	RBS		
A	W	BBa_B0031	RBS		
A	W	BBa_B0032	RBS		
A	W	BBa_B0033	RBS	RBS.4 (weaker)	11
A	W	BBa_B0034	RBS	RBS (Elowitz 1999)	12

parts.mit.edu

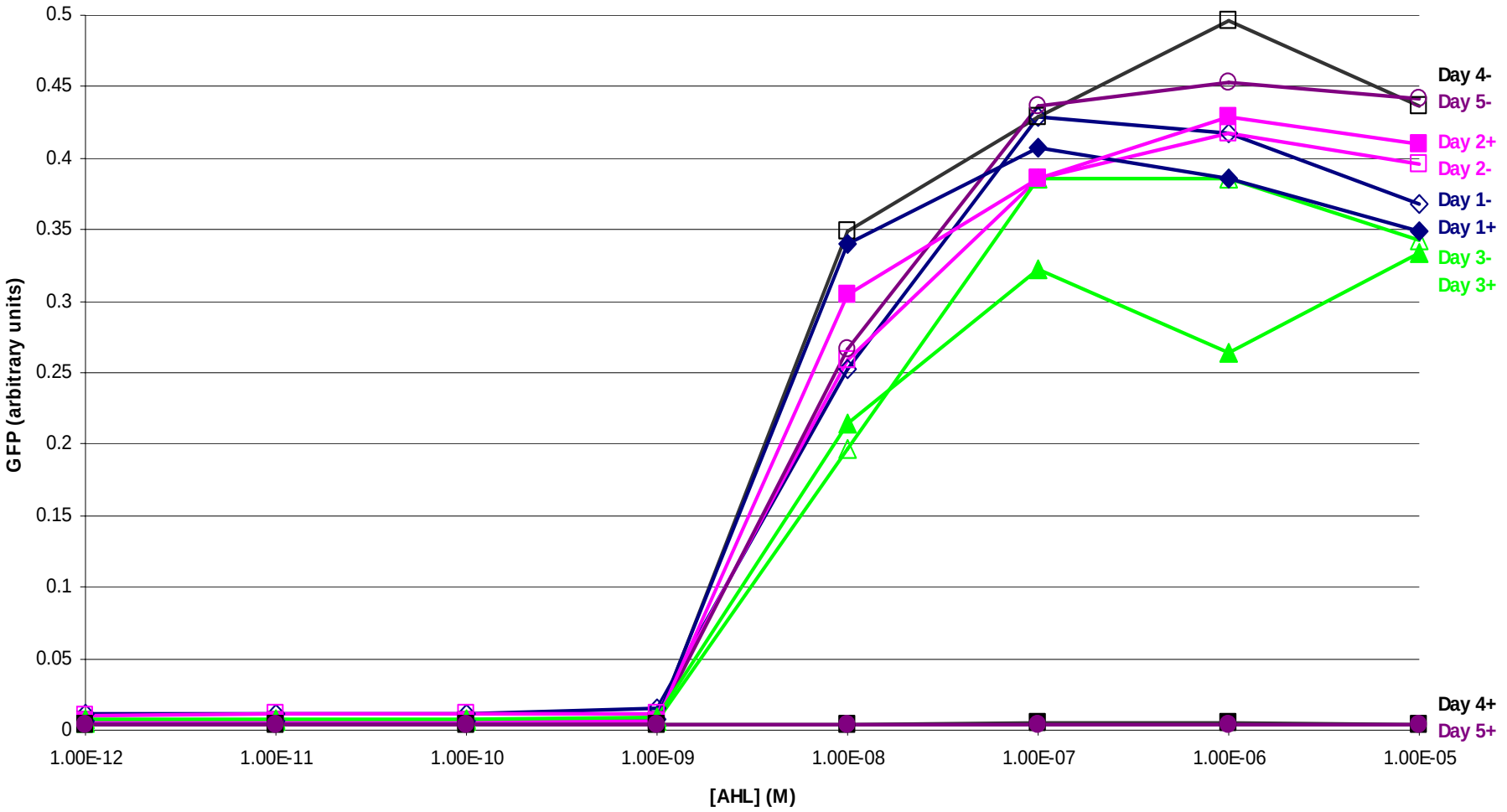
Experimental Protocol



Performance Stability – multi well fluorimetry



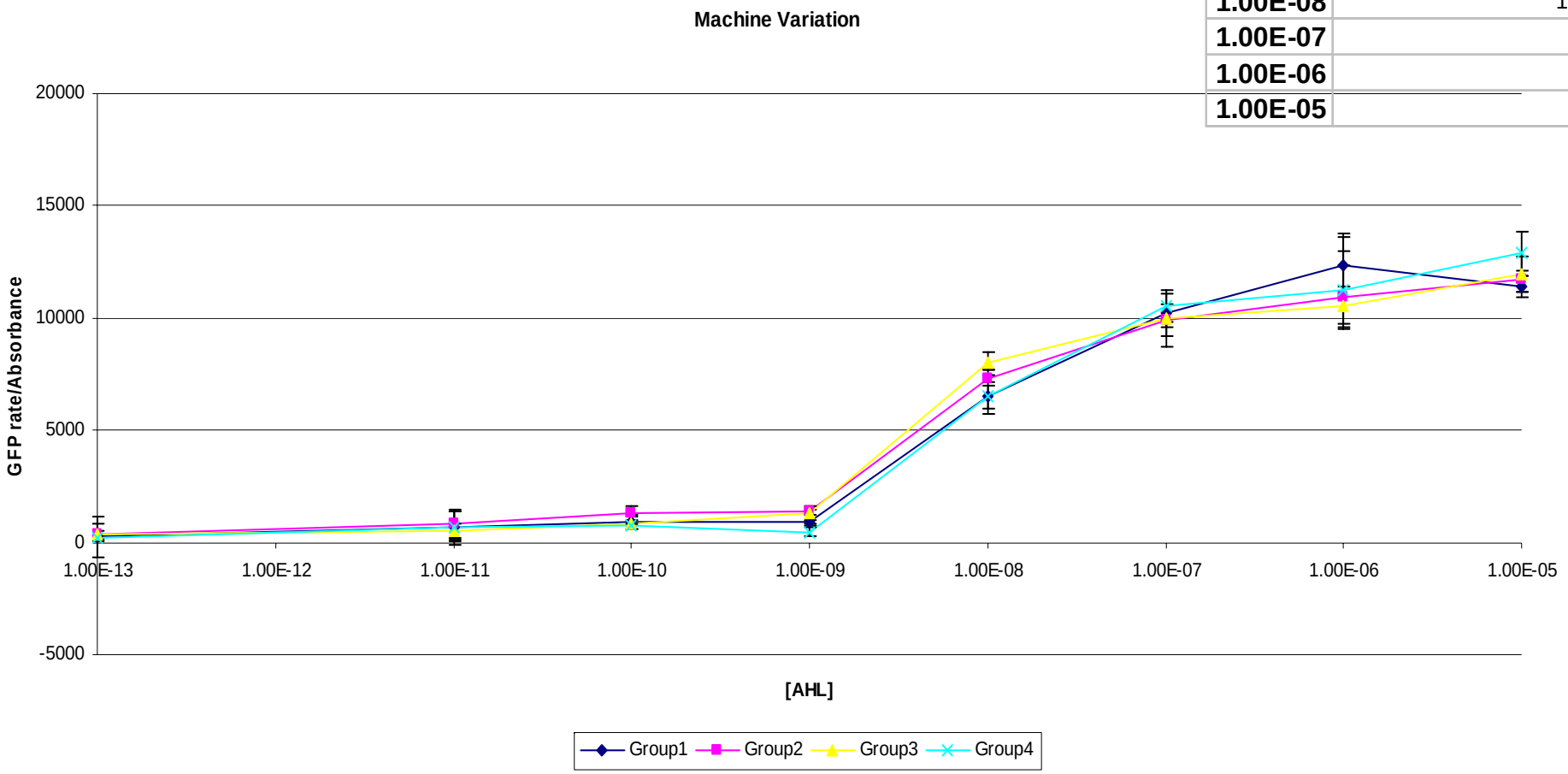
Performance Stability – FACS



T9002

Machine Variation

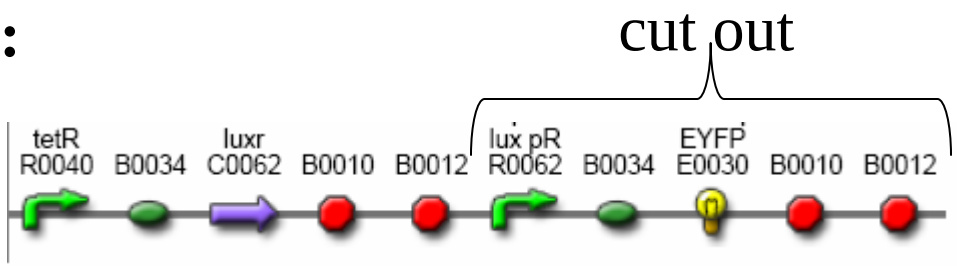
	coeffcient of variation
1.00E-13	22
1.00E-11	18
1.00E-10	26
1.00E-09	41
1.00E-08	10
1.00E-07	3
1.00E-06	7
1.00E-05	5



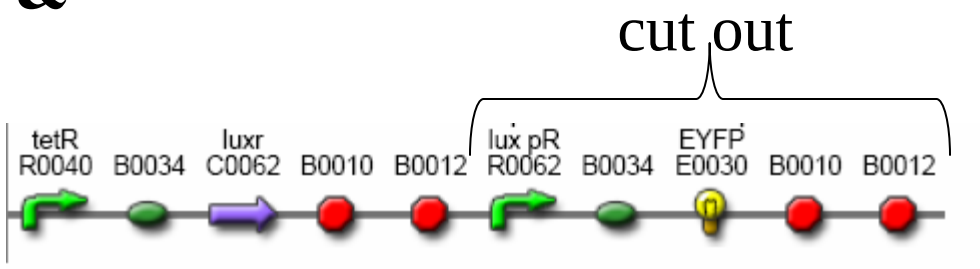
Genetic Stability

I13273

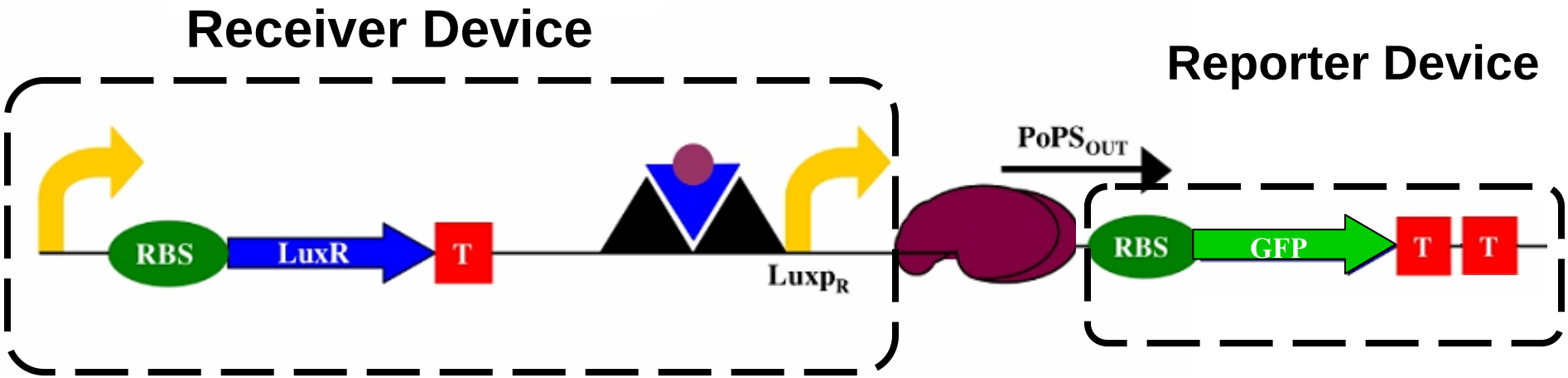
- **Day1, Day 2: No mutations**
- **Day3: No data available**
- **Day4:**



- **Day5:**
  - **Point mutation in C0062 (luxR)**
  - **&**



Genetic Stability

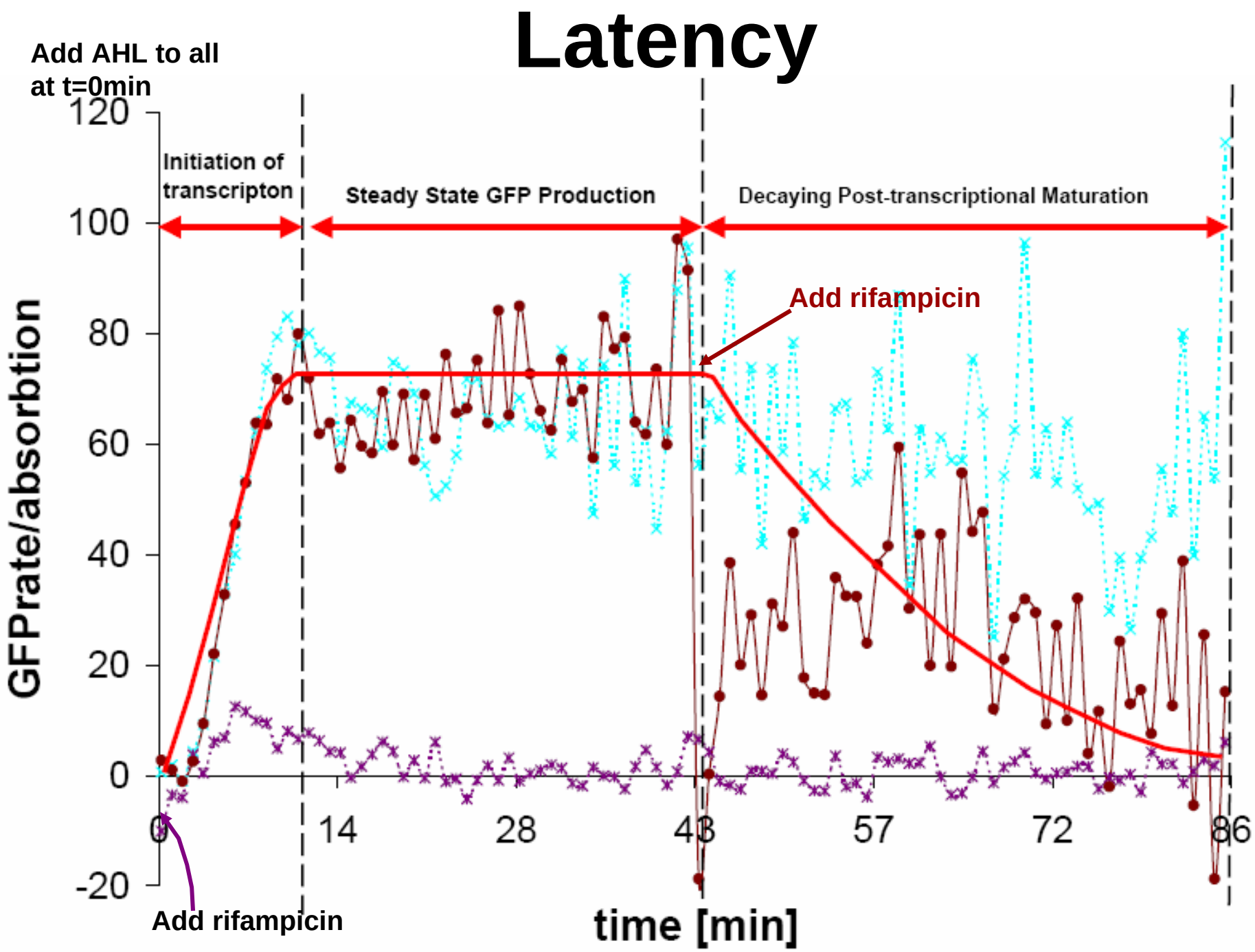

Receiver Device
Reporter Device
PoPS$_{OUT}$
RBS
LuxR
T
Luxp$_R$
RBS
GFP
T
T

PoPs

Relevant Device Characteristics

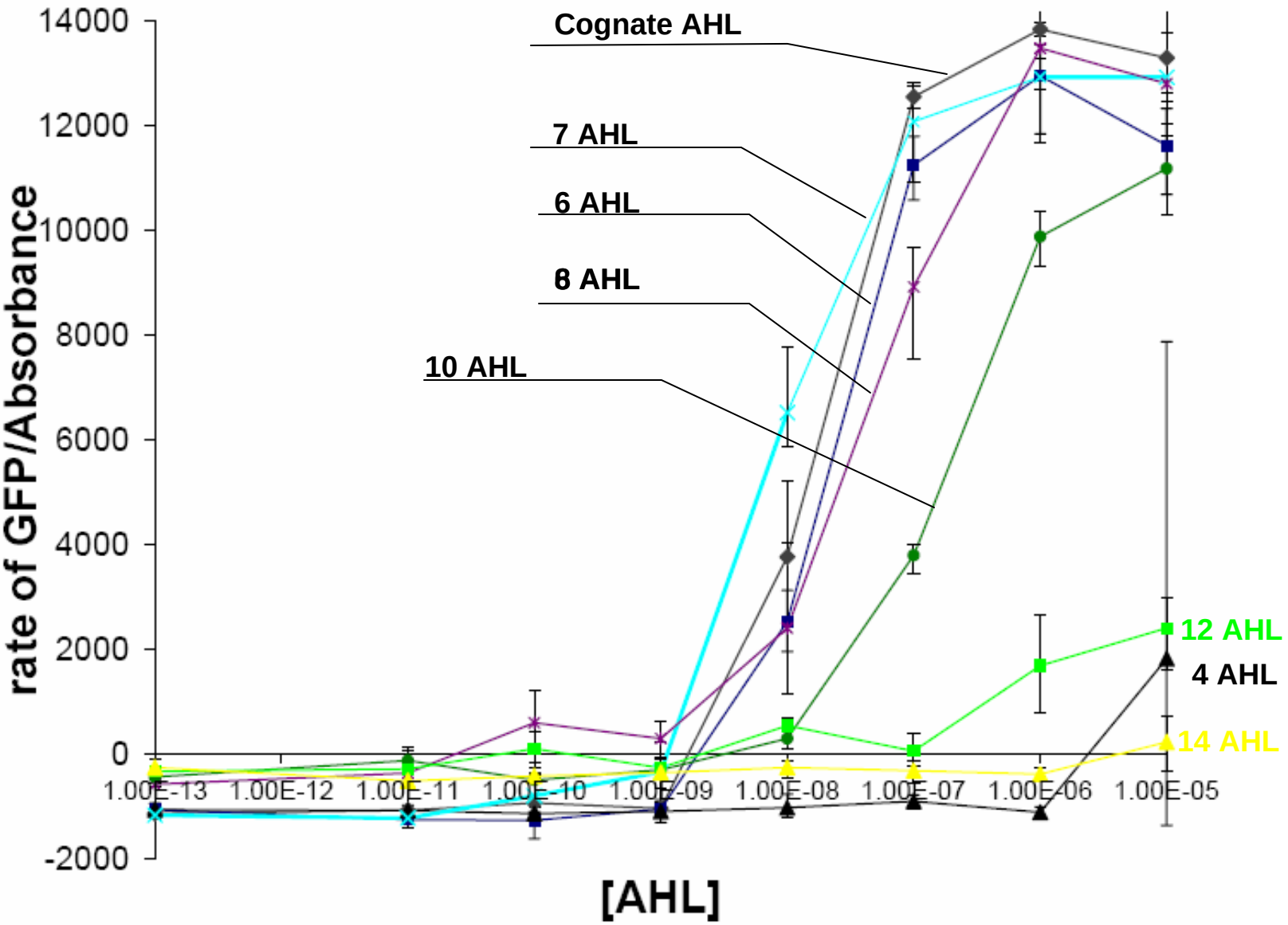
- Transfer Function
- Variability
- Specificity (with respect to inducer)
- Latency (High/Low)
- Stability (genetic and performance)

Latency


Add AHL to all
at t=0min
Initiation of
transcripton
Steady State GFP Production
Decaying Post-transcriptional Maturation
Add rifampicin
Add rifampicin
GFPrate/absorbtion
time [min]
120
100
80
60
40
20
0
-20
0
14
28
43
57
72
86

Carry Over Slides

Specificity



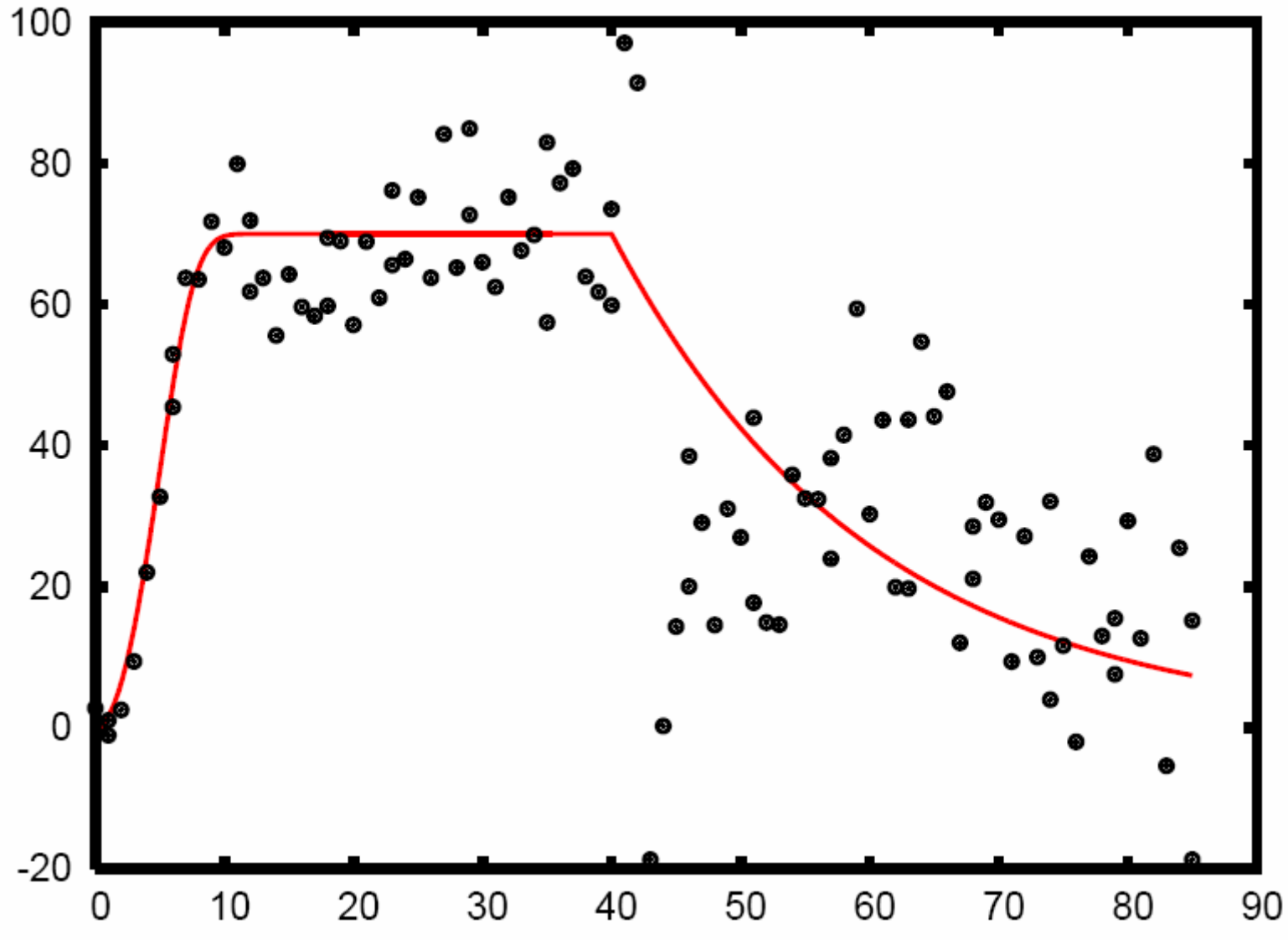
Variability - Overview

- **What difference in performance of device can I expect if from a plate/glycerol and I use it?**
- **Take 8 random colonies from the plate and measure difference in performance**
- **Sequencing to see genetic variation**

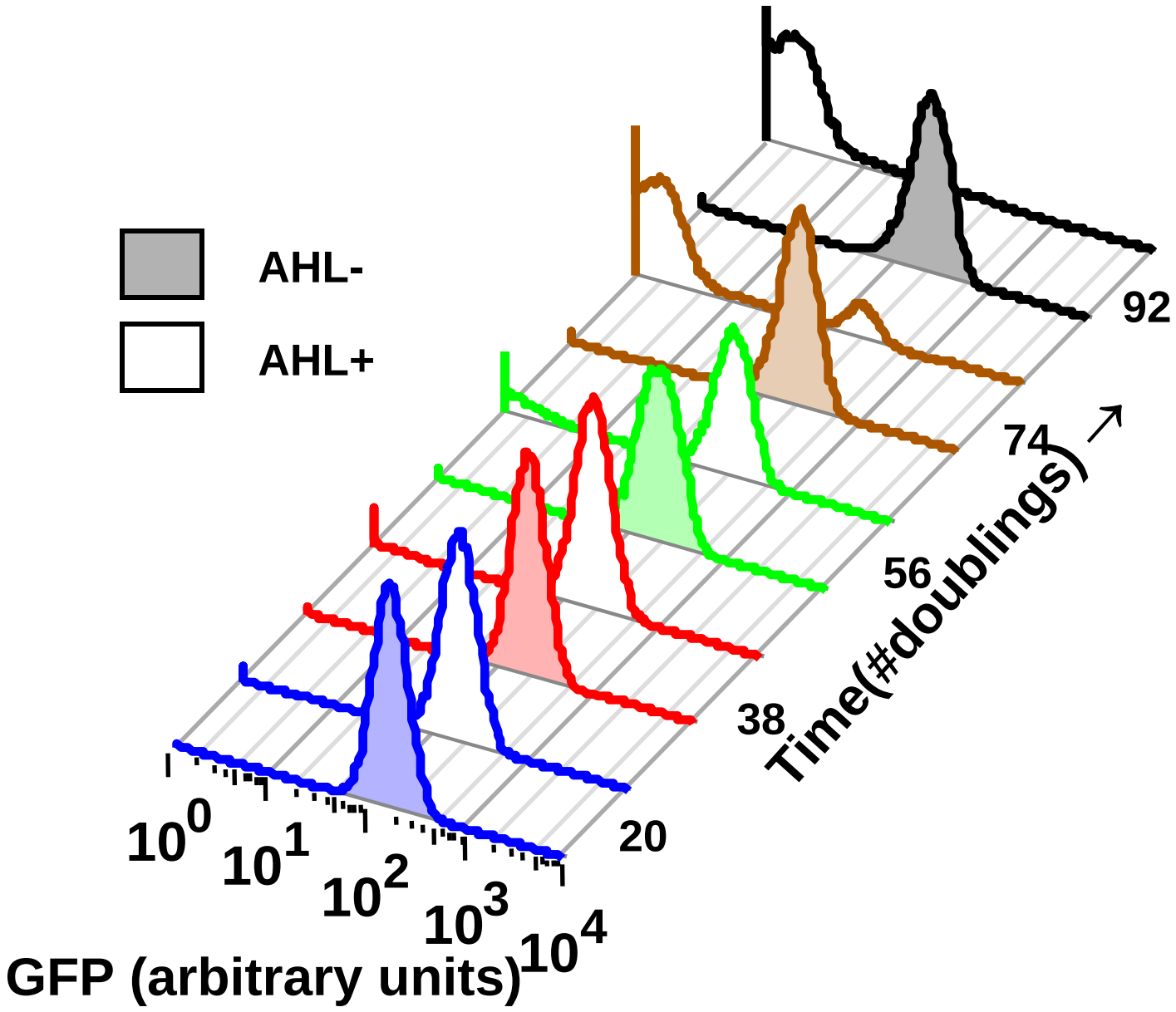
Motivation

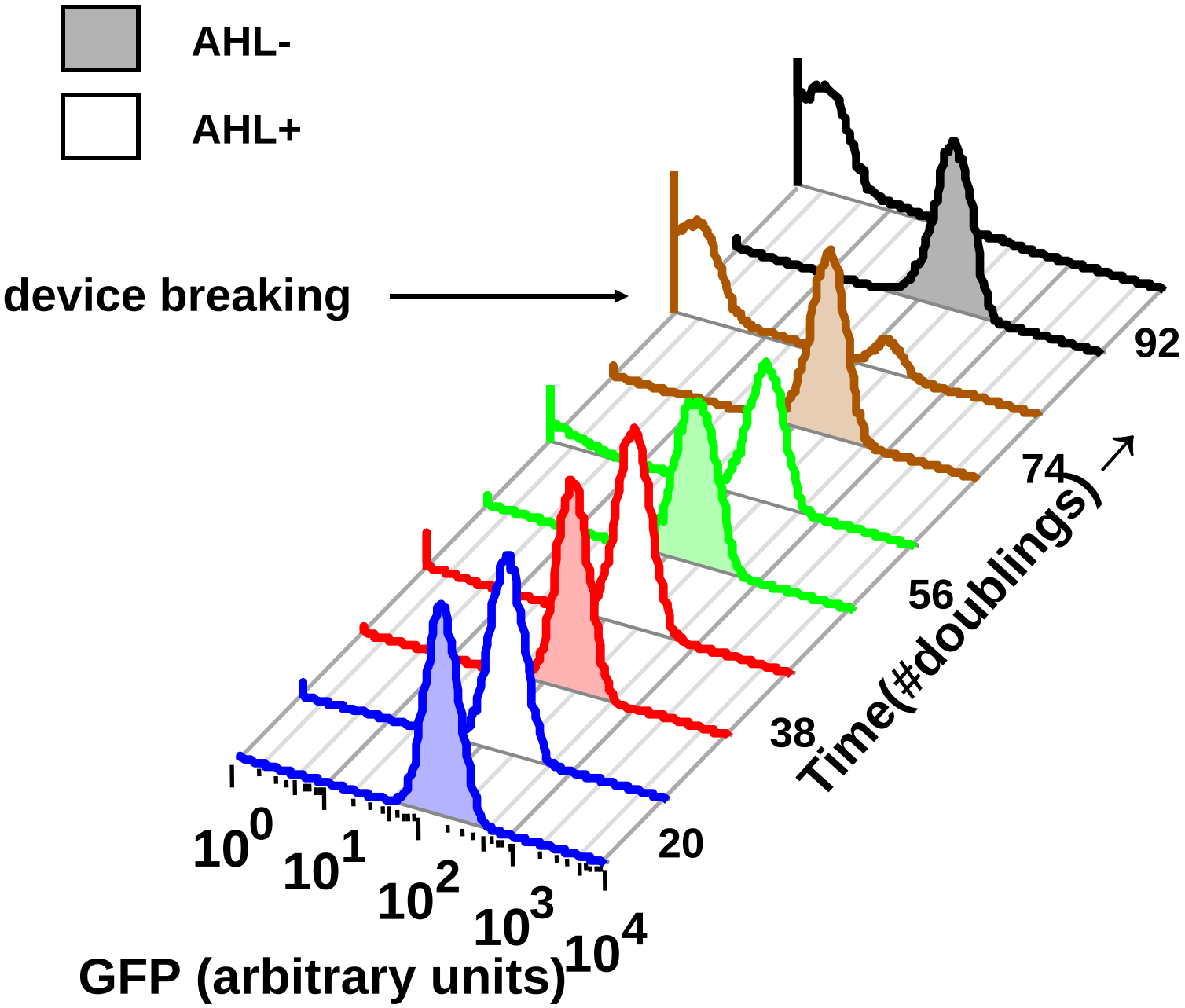
- Design and modeling of more complex systems
- Reliable & specified devices
- Organizing the parts
- Comparison between institutions

Aim: **Define part characterization by example characterization**
Insight into cell-cell communication


100
80
60
40
20
0
-20
0
10
20
30
40
50
60
70
80
90

PoPs


AHL-
AHL+
92
74
56
38
20
Time(#doublings)
10^0
10^1
10^2
10^3
10^4
GFP (arbitrary units)


AHL-
AHL+
device breaking
92
74
56
38
20
Time(#doublings)
10^0
10^1
10^2
10^3
10^4
GFP (arbitrary units)

Stability of I13273

Measure the performance and genetic stability of the receiver

-Under two conditions:

    -Full load

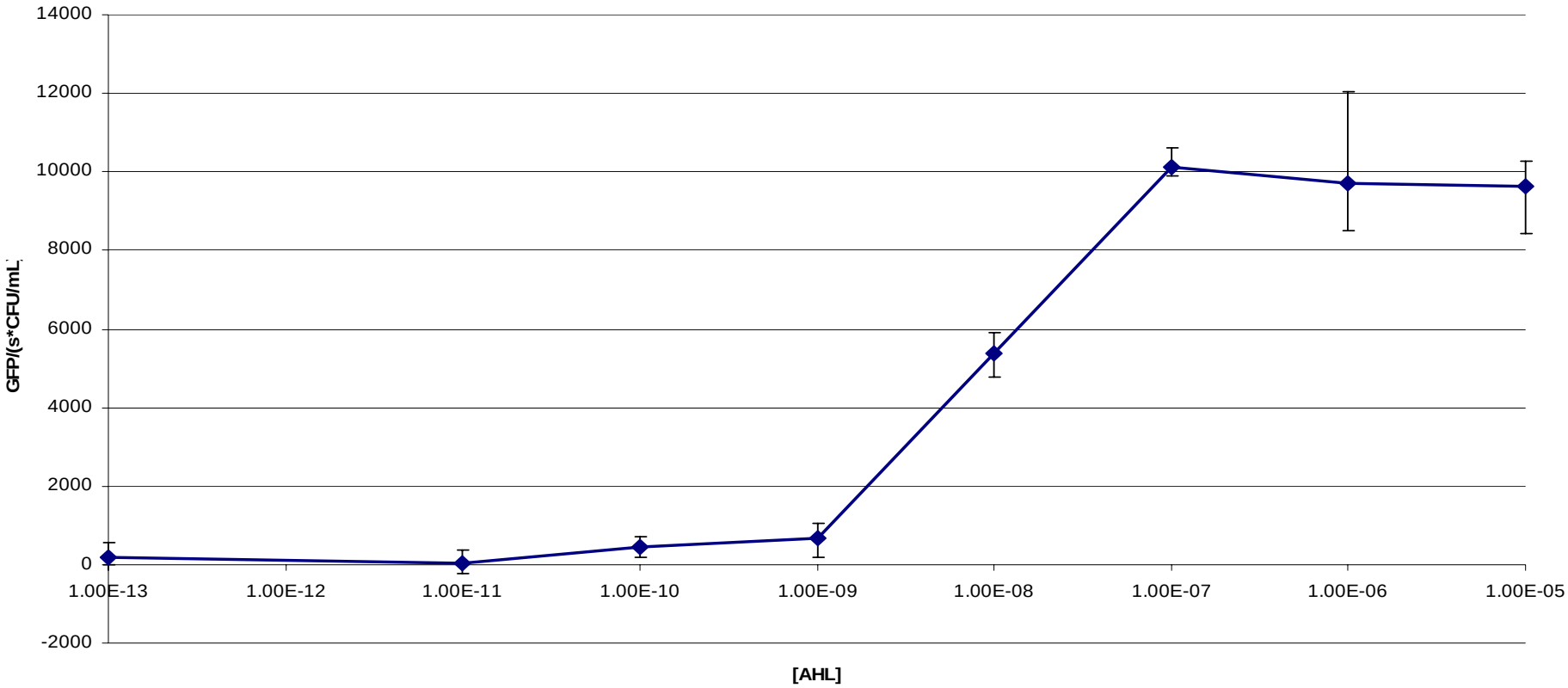
    -No load

-Type of performance degradation (all or nothing vs. graded)

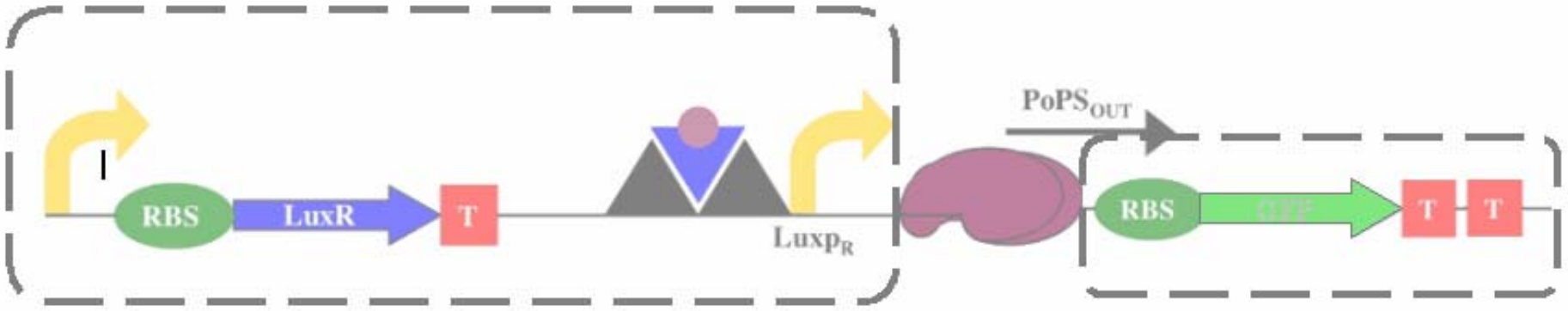
- Cell physiology over time (microscopy)

T9002

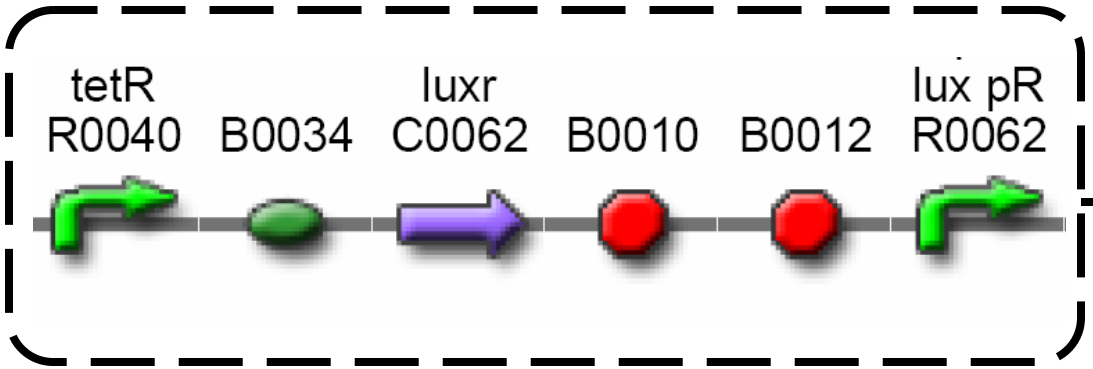
TRANSFER FUNCTION



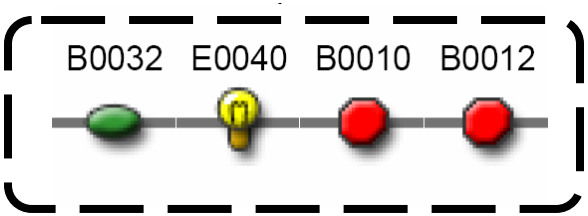
Note: y-axis units are rate of change of relative GFP level per CFU/mL



Receiver Device



Reporter Device



Future Work

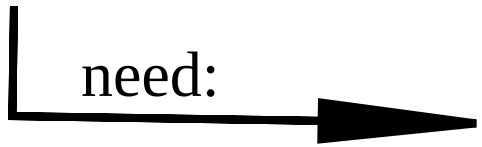
- Latency, Specificity, Variability
  - Repeat to avoid human mistakes
  - Measure reporter latency and use it to estimate the latency purely of receiver device (stop translation exp)
  - Calibrate output in PoPs
- Stability
  - Fine tune protocol
  - Repeat for GFP
  - Get microscope images
  - Repeat with more stable/selectable reporter?

Motivation

Synthetic Biology

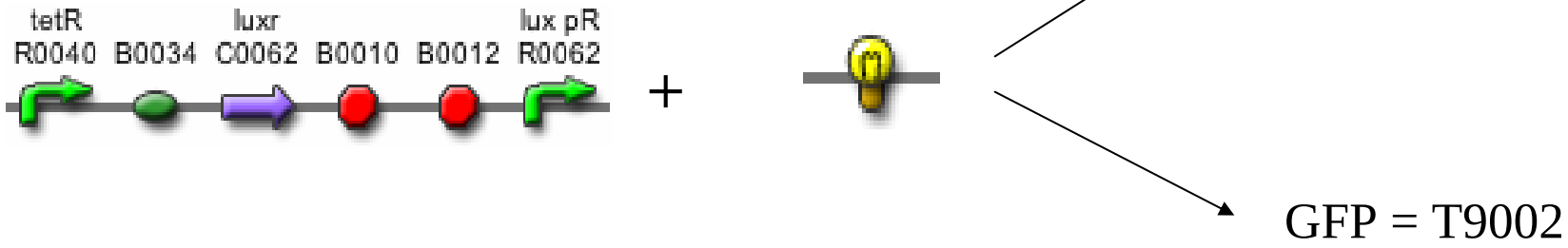
Building systems → robust, reusable, self-replicating

Medical → antibiotic/enzyme production , *in vivo* monitoring devices
Engineering → information processing (logic gates, repressilator)
Science → new perspective to understand the living world, intracellular physics

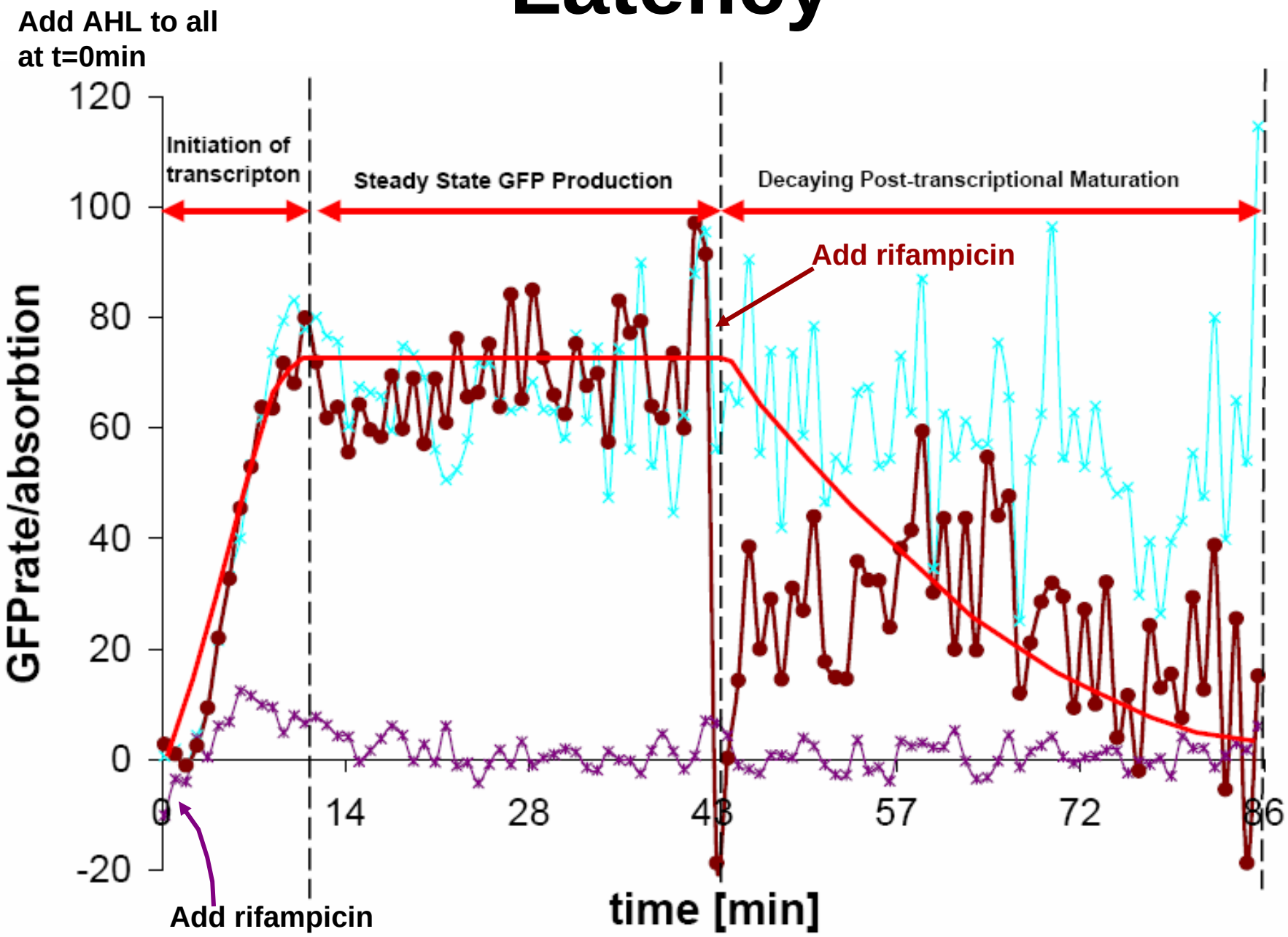


Reliable & specified parts

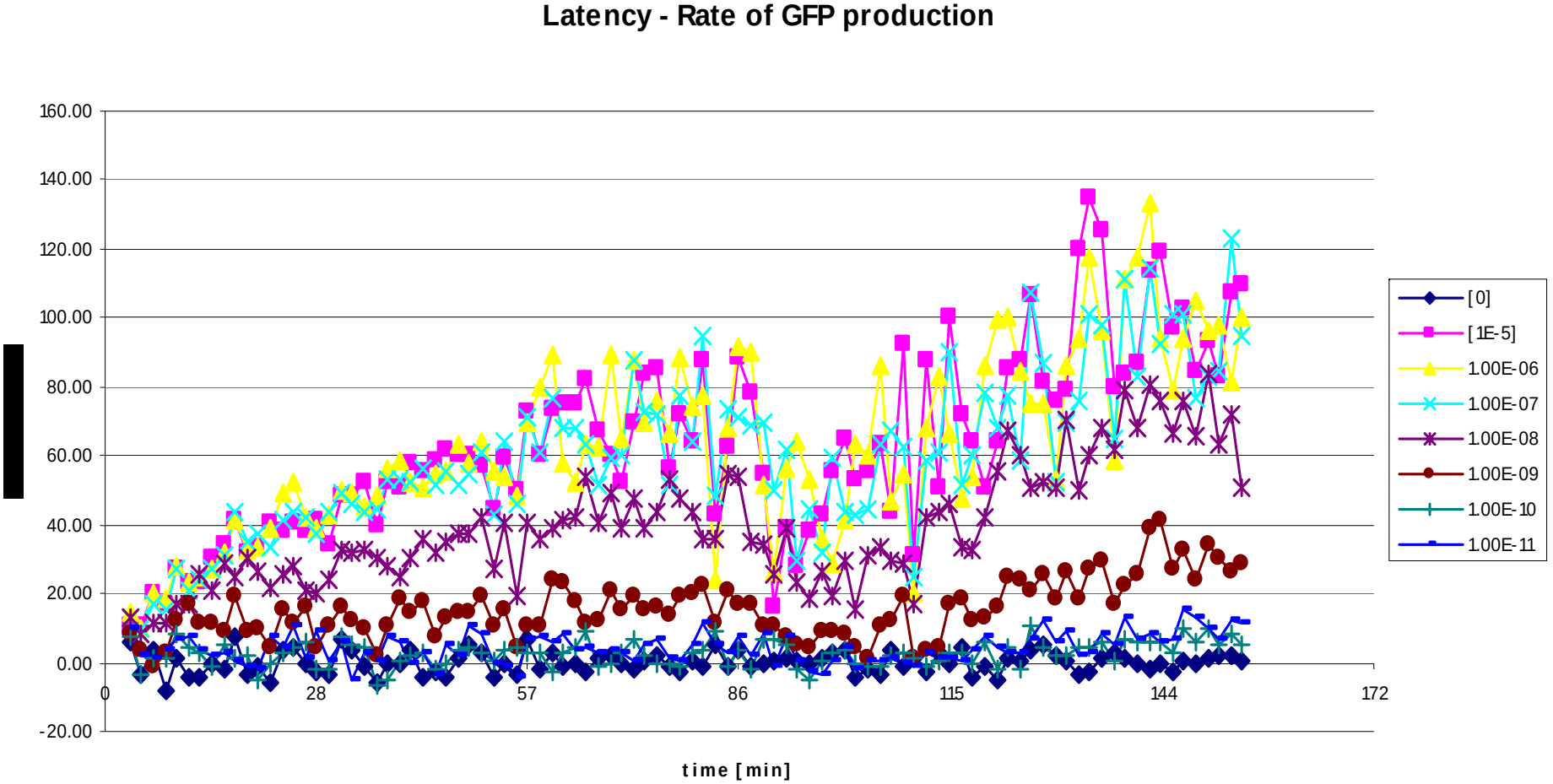
Aim: **Define part characterization by example characterization**
Insight into cell-cell communication


tetR
R0040
B0034
luxr
C0062
B0010
B0012
lux pR
R0062
+
YFP = I13273
GFP = T9002

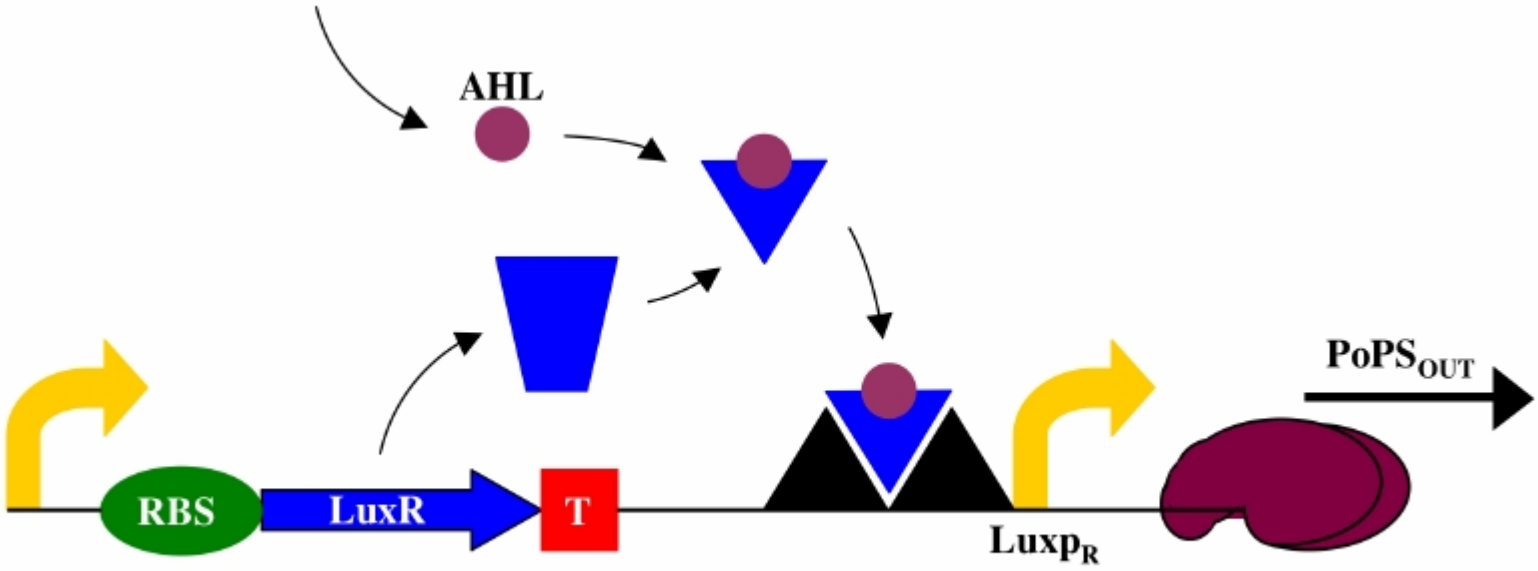
Latency


Add AHL to all
at t=0min
Initiation of
transcripton
Steady State GFP Production
Decaying Post-transcriptional Maturation
Add rifampicin
GFPrate/absorbtion
120
100
80
60
40
20
0
-20
0
14
28
43
57
72
86
time [min]
Add rifampicin

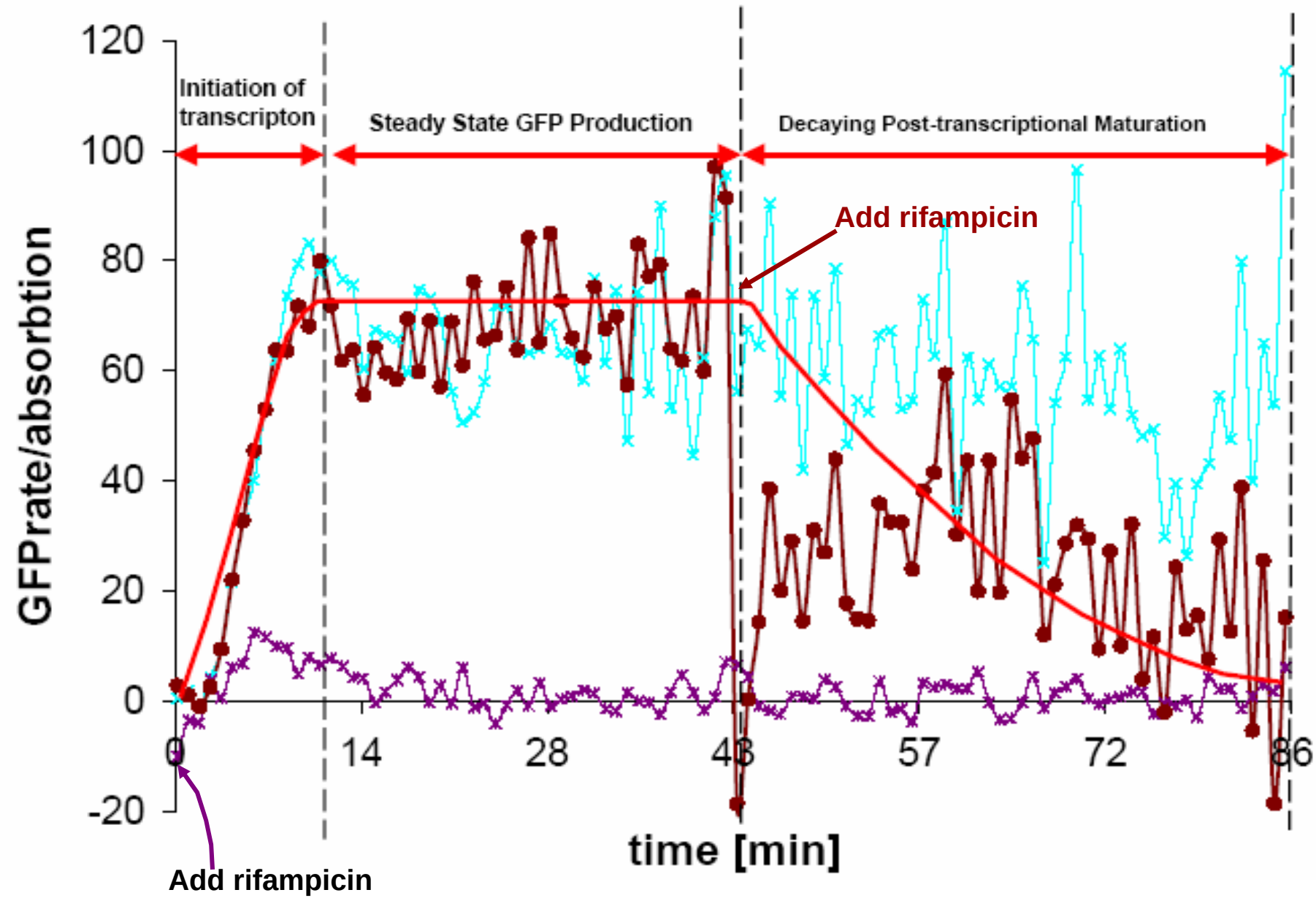
T9002


Latency - Rate of GFP production
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
-20.00
0
28
57
86
115
144
172
time [min]
[0]
[1E-5]
1.00E-06
1.00E-07
1.00E-08
1.00E-09
1.00E-10
1.00E-11

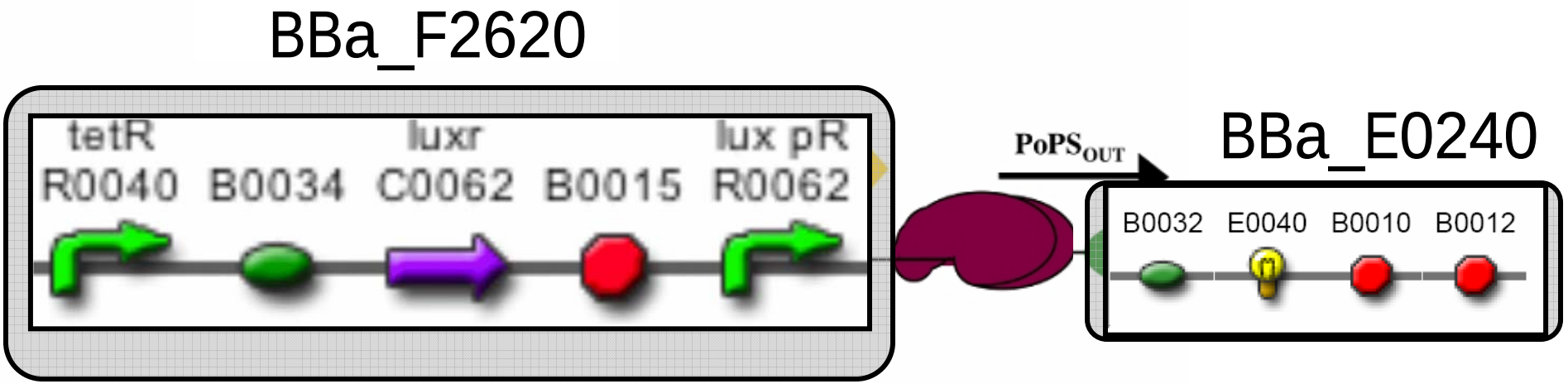
Lux Receiver



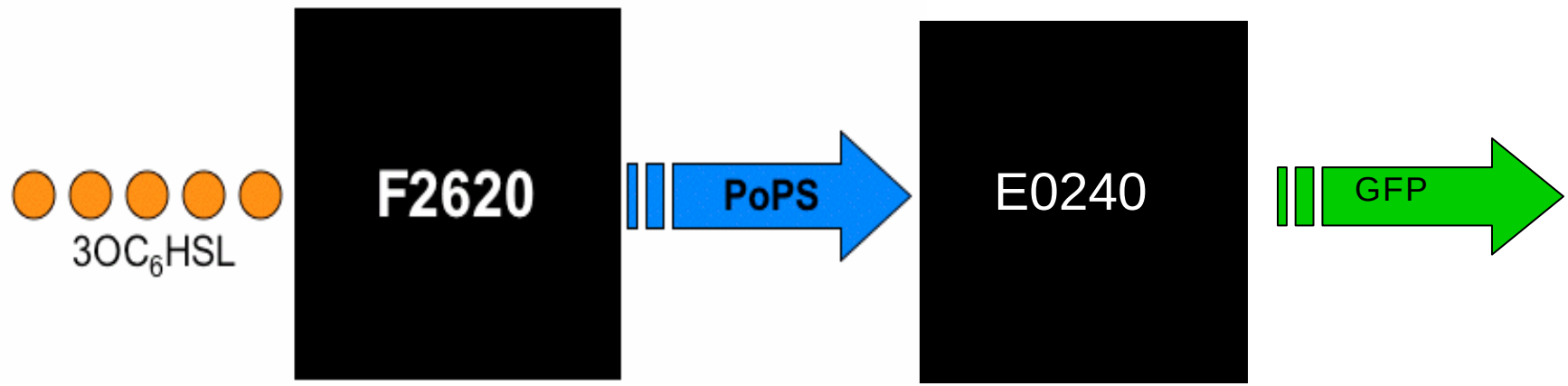
Latency

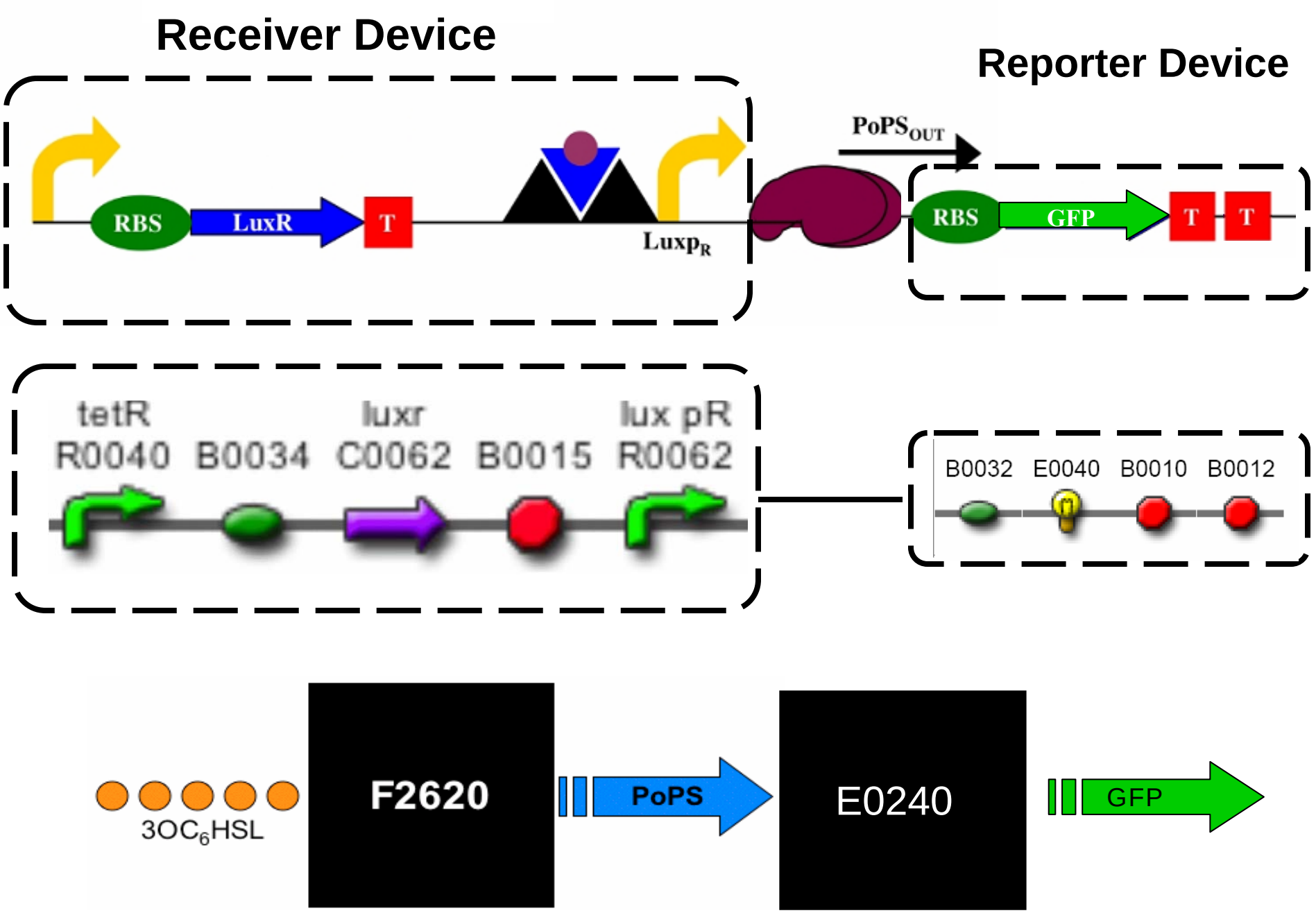

Add AHL to all
at t=0min
Initiation of
transcripton
Steady State GFP Production
Decaying Post-transcriptional Maturation
Add rifampicin
GFPrate/absorbtion
120
100
80
60
40
20
0
-20
0
14
28
43
57
72
86
time [min]
Add rifampicin

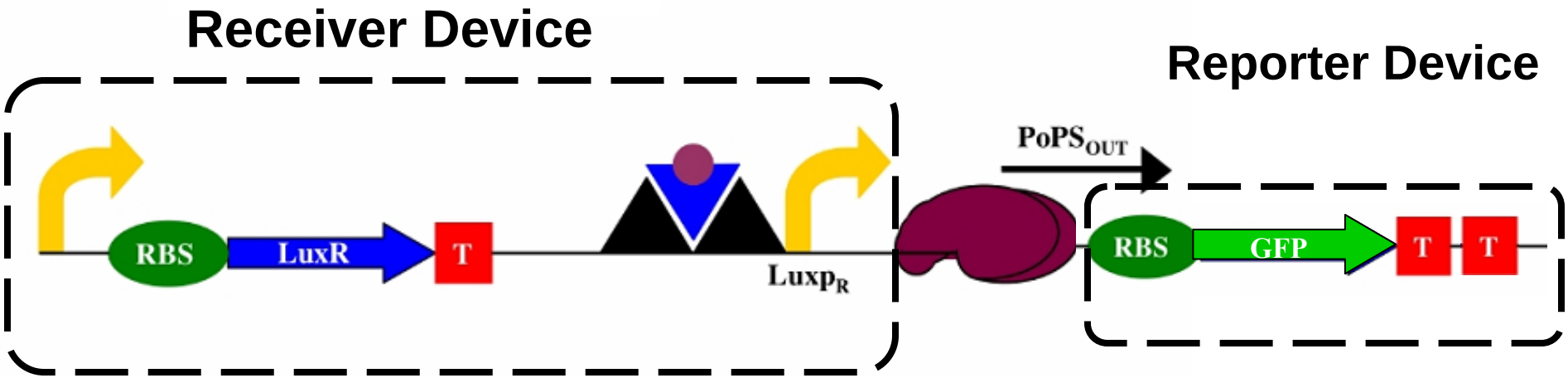
Cell-Cell Communication Device

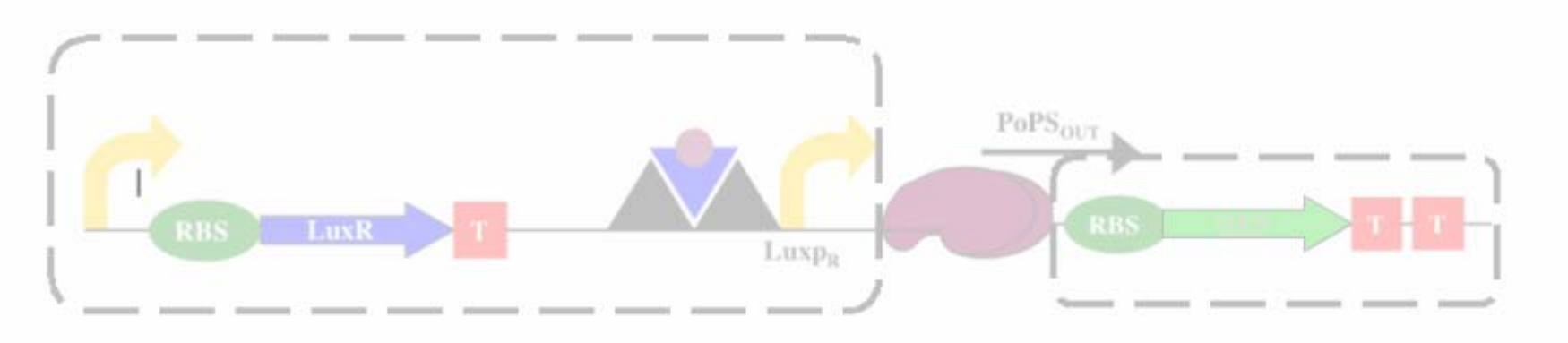


Cell-Cell Communication Device

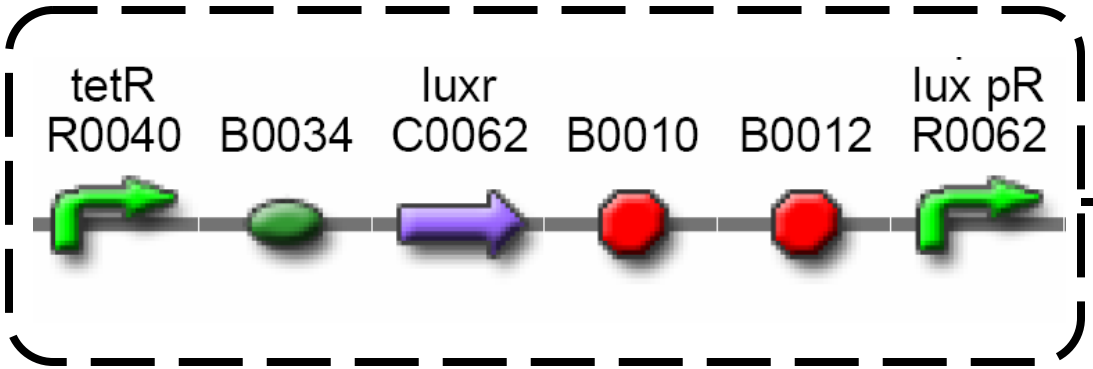



Receiver Device
Reporter Device
RBS
LuxR
T
Luxp$_R$
PoPS$_{OUT}$
RBS
GFP
T
T
tetR
R0040
B0034
luxr
C0062
B0015
lux pR
R0062
B0032
E0040
B0010
B0012
3OC$_6$HSL
F2620
PoPS
E0240
GFP

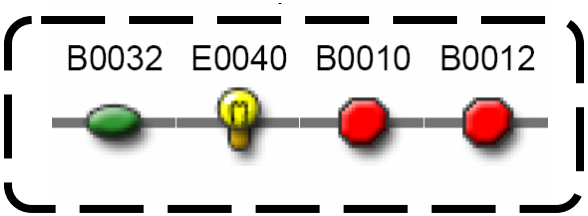

Receiver Device
Reporter Device
PoPS$_{OUT}$
RBS
LuxR
T
Luxp$_R$
RBS
GFP
T
T

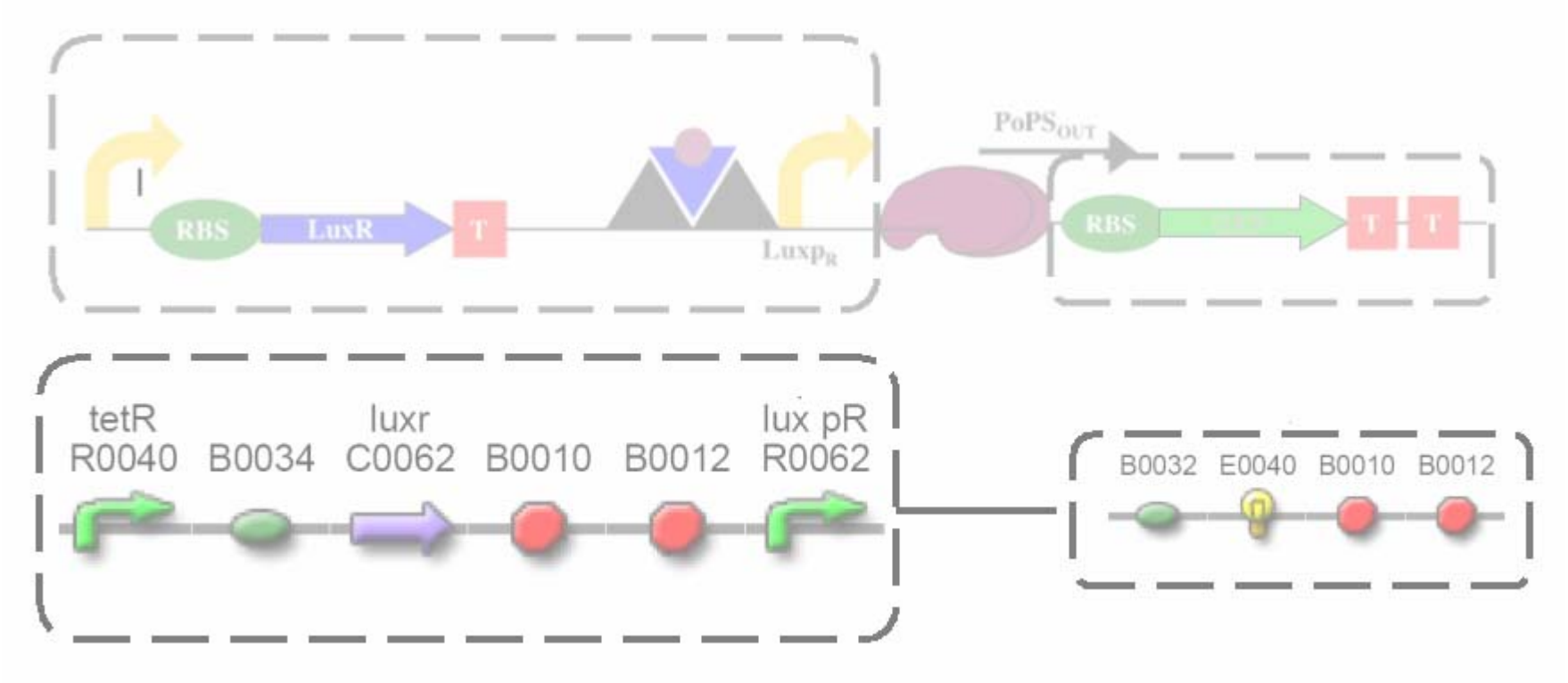


Receiver Device



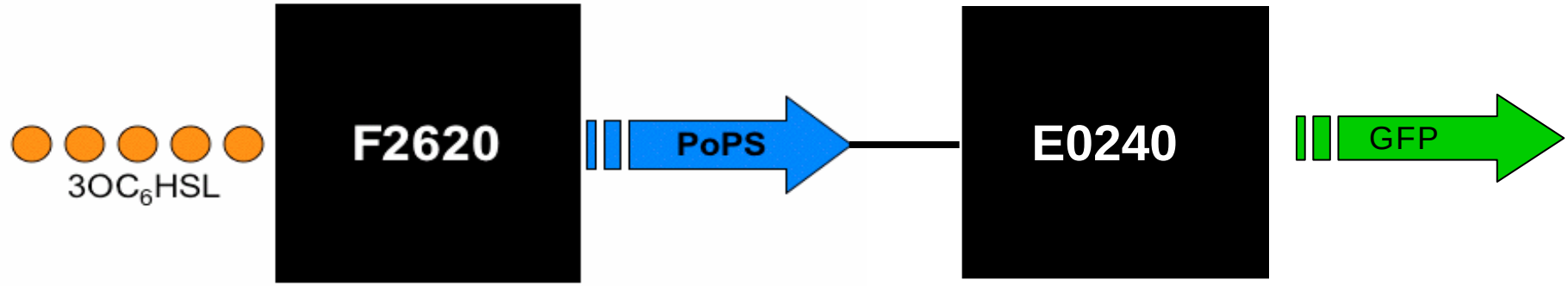
Reporter Device



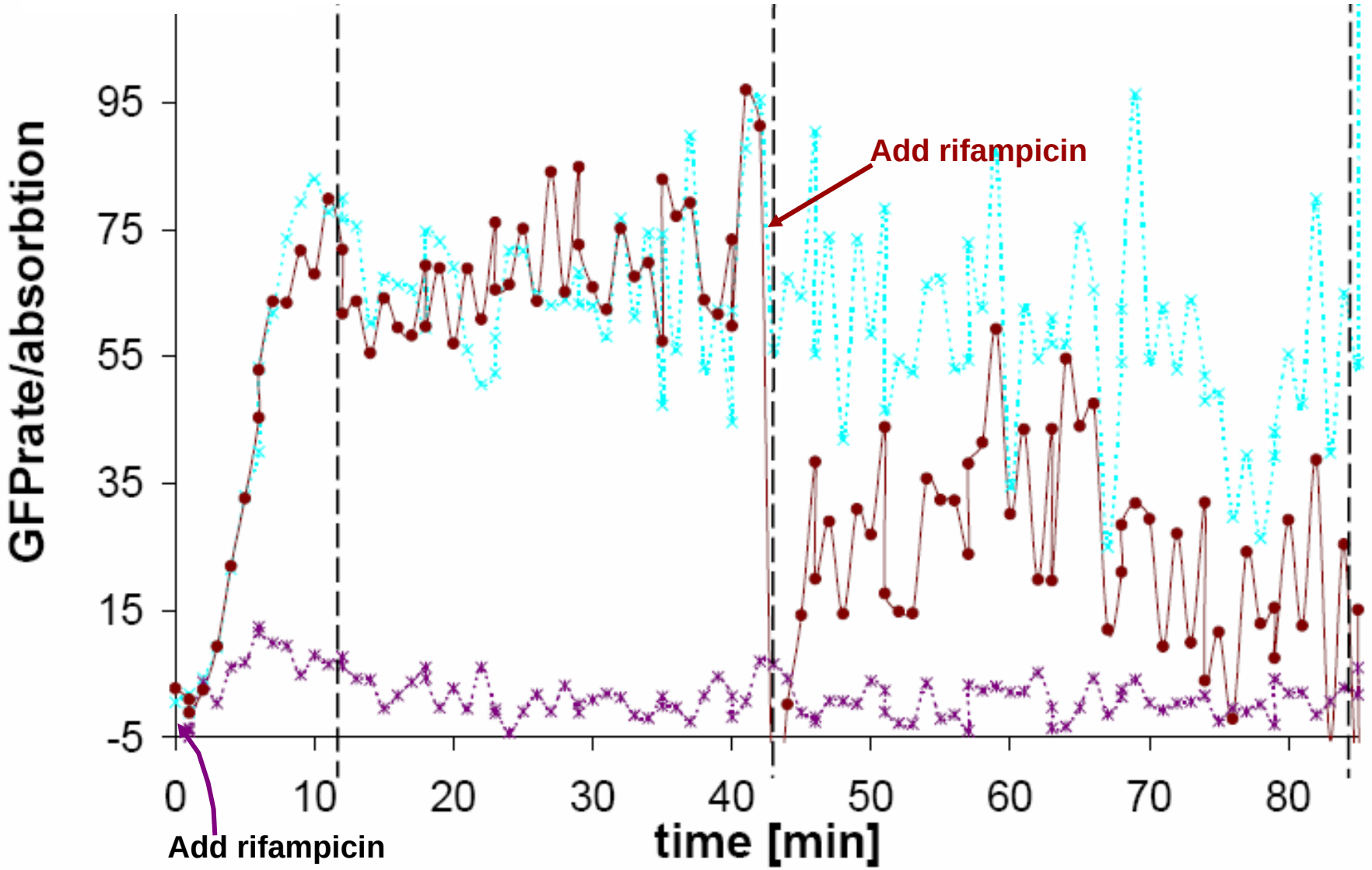


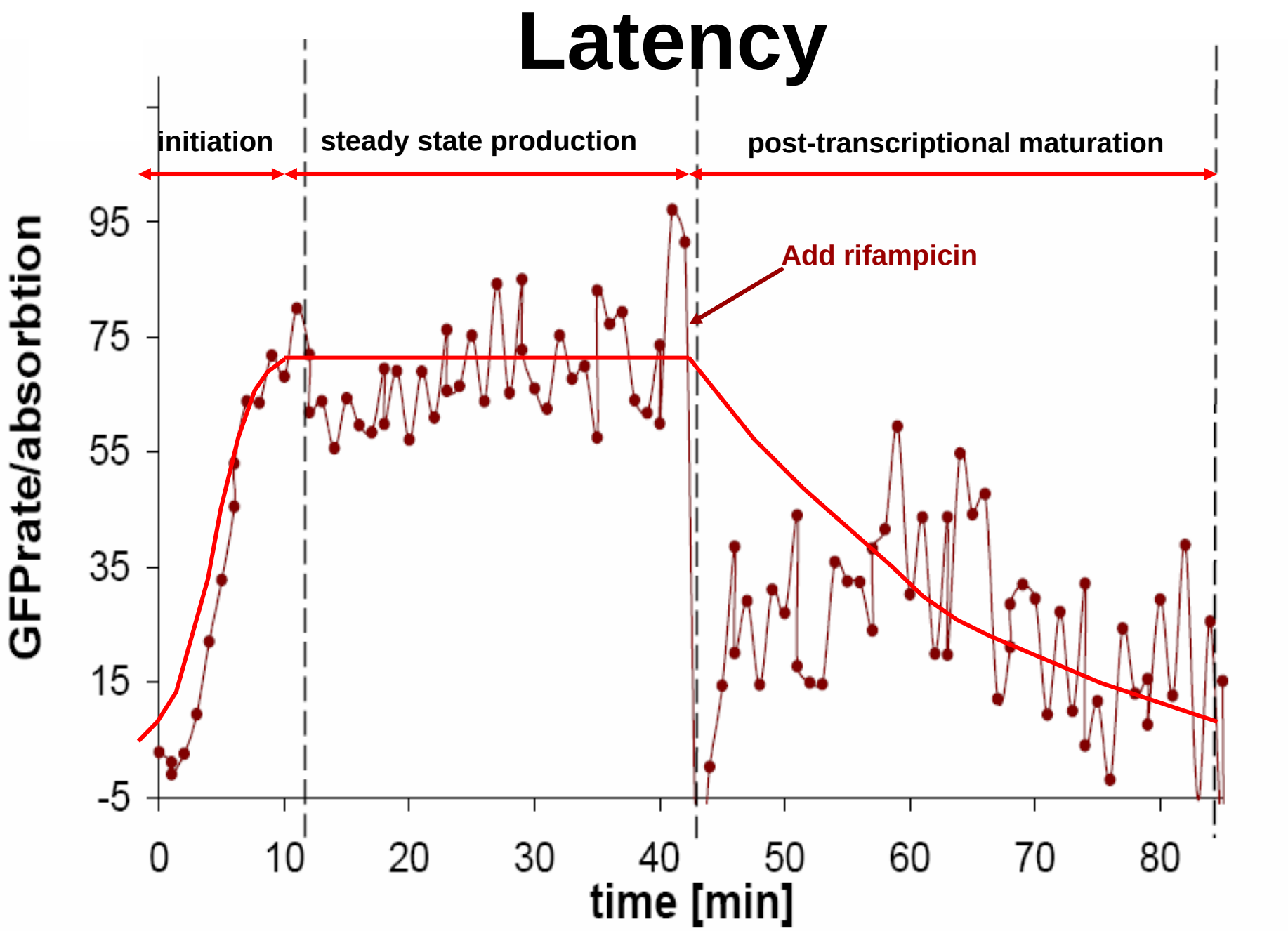
Receiver Device

Reporter Device



Latency


Add AHL to all
at t=0min
Add rifampicin
Add rifampicin
GFPrate/absorbtion
95
75
55
35
15
-5
0
10
20
30
40
50
60
70
80
time [min]


Latency
initiation
steady state production
post-transcriptional maturation
Add rifampicin
GFPrate/absorbtion
95
75
55
35
15
-5
0
10
20
30
40
50
60
70
80
time [min]